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ABSTRACT

Title of Thesis: "Effects of Nicotine Administration, Cessation, and Differential Housing Conditions on Aggressive Behaviors of Male and Female Rats"

Author: Peter M. Scheufele, Master of Science, 1997

Thesis directed by: Neil E. Grunberg, Ph.D.
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The present experiment examined effects of nicotine administration, nicotine cessation, and two housing conditions (individual vs. grouped) on social interaction and serum testosterone in Long-Evans rats. Nicotine reduced aggressive behaviors of male and female rats, particularly in single-housed conditions. Effects of nicotine on serum testosterone in males also depended on housing condition with nicotine reducing serum testosterone of single-housed males, but not group-housed males. In addition, single-housed males and females exhibited more aggressive behaviors than group-housed animals. Male testosterone levels and aggressive behaviors returned to baseline during nicotine cessation. The results suggest that effects of nicotine may be modified by environmental or social situations. Further, for male subjects, aggressive behaviors were accounted for by housing and drug conditions rather than by testosterone levels. The results suggest that changes in the effects of nicotine because of environmental or social conditions may contribute to changes in smoking behaviors and behaviors of smokers.

Effects of Nicotine Administration, Cessation, and

Differential Housing Conditions on

Aggressive Behaviors of Male and Female Rats

by

Peter M. Scheufele

Masters Thesis submitted to the Faculty of the

Department of Medical and Clinical Psychology

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INTRODUCTION

Cigarette smoking-related illnesses account for over 400,000 deaths per year in the United States, more than the number of deaths from AIDS, alcohol, car accidents, murders, suicides, and drugs combined (Lynch & Bonnie, 1994). This statistic is particularly tragic because the causal relationship between cigarette smoking and subsequent serious illnesses have been well documented (Grunberg, Brown, & Klein, 1997), yet people continue to smoke because cigarettes and other forms of tobacco are addicting (USDHHS, 1988). Nicotine is the primary pharmacologic agent in tobacco that causes this addiction. Nicotine has rewarding effects that may be mediated via dopaminergic release from the ventral-tegmental in the nucleus accumbens in the brain (Grunberg et al., 1997). Nicotine has additional effects that contribute to tobacco use and make abstinence difficult. For example, cigarette smokers report that smoking helps them to focus their thoughts, to cope with stress, and to keep their body weight under control (Grunberg et al., 1997; USDHHS, 1988). These effects contribute to the difficulty that some smokers have quitting.

Smoking cessation also results in craving, restlessness, irritability, depression, and sleep disturbances (Hughes, Higgins, & Hatsukami, 1990; Acri & Grunberg, 1992). Some of these effects experienced by smokers attempting to quit may modify their social interactions with others. Significantly, smokers attempting to quit sometimes engage in more hostile or aggressive social interactions, such as having fights with their spouses (Schachter et al., 1977).

The possibility that humans may become less aggressive with nicotine and

more aggressive during acute nicotine abstinence has been investigated in a few studies (Schechter & Rand, 1974; Cherek, 1981; Cherek, Bennett, & Grabowski, 1991). These studies report that smokers administer more "aggressive responses" under controlled laboratory conditions in an inverse dose-response relationship with nicotine, and a positive dose-response relationship with nicotine abstinence. However, "aggressive responses" were operationally defined by Cherek (1981) and colleagues (1991) as bar pressing on a lever that took money away from another subject who actually did not exist. Schechter and Rand (1974) defined aggression as increasing the amount of shock (that really was not administered) to another person who was a confederate of the experimenter. These reports suggest that nicotine and nicotine abstinence are related to aggressive behaviors, but they did not measure aggressive behaviors of smokers in the context of social interactions, nor did they compare the amount of aggressive responses displayed by smokers to those exhibited by non-smokers. In addition, it is not certain that these operationalizations are true indices of aggression rather than measures of other behaviors, such as competition and obedience.

Animal research has proven to be useful to study effects of nicotine, including behavioral and biological variables. Much of this animal research has generalized to human smokers. For example, the relationship of weight gain following nicotine abstinence observed in humans has been examined using animal studies, and many findings with animals helped to guide human studies (e.g., Grunberg, 1982; Winders & Grunberg, 1989; Grunberg, 1992). Nicotine self-administration has paralleled human studies of smoking, but the animal

studies have allowed complete control of the independent variable and detailed analyses of the behavioral and biological variables (Corrigall & Coen, 1989; Cox, Goldstein, & Nelson, 1984). Animal studies are particularly useful to study the effects of nicotine on aggressive behaviors, because animals exhibit predictable and characteristic aggressive behaviors that can be measured under controlled laboratory conditions. In addition, animal studies examining the effects of drugs on aggressive behaviors are useful for ethical and logistic reasons.

Animals exhibit many forms of aggression, allowing the study of this complex behavior in animals under controlled conditions in order to develop hypotheses about human aggression (Manning & Stamp Dawkins, 1992). Many forms of rodent aggression exist and have been studied, including offensive, defensive, predatory, and maternal (Albert, Jonik, & Walsh, 1992). Aggression in rats can be initiated through a number of experimental conditions including isolation, cohabitation with a conspecific of the opposite sex, sexual experience, pregnancy and lactation, competitive experience, or repeated exposure to unfamiliar conspecifics. Aggressive behaviors have been measured in rats that were not exposed to any prior experimental treatment (e.g., Christie & Barfield, 1979). However, the level of aggression measured was substantially less than that of males cohabitating with females (Albert et al., 1992). In addition, strain differences exist in the relative levels of aggression as a result of selective breeding by researchers who develop highly aggressive strains of animals to study behavioral, neuronal, and genetic differences between high- and low-aggression strains (Manning & Stamp Dawkins, 1992).

Despite differences in type, amount, and experimental conditions that have

been found to initiate it, animal aggression consists of characteristic behaviors that can be observed and quantified. For example, rats exhibit characteristic aggressive behaviors that are easily identified and measured (Blanchard & Blanchard, 1977; Koolhaas, Schuurman, & Wiepkema, 1980). These consist of a wrestling-type lunge toward a conspecific from a normal upright position on all four paws which may or may not result in the attacker biting the defender, and "boxing", in which a rat pushes a conspecific with its front paws from a reared upright position standing on its back paws. (For examples of these behaviors, see Appendix I.) Levels of aggression are determined by counting the amount, duration, or latency to display characteristic aggressive behaviors displayed by experimental subjects during an experimental period. Whereas aggressive behaviors can be measured in albino as well as non-albino rodent strains, a review of the literature indicates that rats of the non-albino Long-Evans strain are often used in rodent social interaction paradigms (e.g., Latané & Werner, 1978; Meaney & Stewart, 1979; Moore, Byers, & Baron, 1981; Primus & Kellogg, 1990). The Long-Evans strain is the result of selectively breeding a Sprague-Dawley albino strain laboratory rat to a wild Norway hooded rat. The resulting Long-Evans rat has distinctive grey coloring on its upper body and grey eyes, as opposed to the all-white coloration and pink eyes of an albino rat.

Studies examining the effects of nicotine on aggressive behaviors with animal subjects parallel findings of human studies. That is, nicotine has been reported to reduce aggressive behaviors exhibited by rodents (Silverman, 1971; Rogers, 1979; Driscoll & Bättig, 1981). However, the methods of these studies make the findings somewhat equivocal with regard to nicotine and social

interactions. Silverman (1971) put rats that had received an injection of nicotine with their cage-mates after a 5 to 6 hour absence from the cage. This procedure was conducted a number of times over a period of weeks. Therefore, this study examined the effects of nicotine only with a familiar conspecific that may or may not generalize to other social interactions. Other studies report that nicotine reduced shock-induced fighting in rats (Rogers, 1979; Driscoll & Bättig, 1981), but aggressive behaviors observed in these studies were induced by an electric shock to the animals' feet rather than in the context of the animals' typical social interactions. Hutchinson & Emley (1973) also reported that nicotine affects aggressive behaviors (in a U-shaped function), but this study used squirrel monkeys and examined aggression toward a physical object, rather than examining aggression in the context of social interactions.

Studies examining aggressive behaviors of rodents defending their home cage against another "intruder" rat, or interacting in a neutral social interaction conditions, report that decreases in serum testosterone of male rats result in decreases in some forms of aggression (Albert, Jonik, Watson, Gorzalka, & Walsh, 1990; Primus & Kellogg, 1990; Albert, Walsh, Gorzalka, Siemens, & Louie, 1986). Interestingly, heavy cigarette smoke resulted in a 54% decrease in serum testosterone of male beagles compared to control animals (Mittler, Pogach, & Ertel, 1983), but this study did not examine aggressive behaviors. In addition, environmental conditions have been reported to affect behavioral and biological responses of animals. Specifically, differential housing conditions resulted in different levels of gregariousness in rats when measured in a social interaction paradigm (Latané, Cappell, & Joy, 1970; Meaney & Stewart, 1979).

Also, male and female rats were reported to have differential responses to individual or crowded housing conditions based on a biochemical measurement (Brown & Grunberg, 1995). Based on these reports, administration and cessation of nicotine may change serum testosterone and social and aggressive behaviors, and, in addition, differential housing conditions may influence social and aggressive behaviors.

There are a number of possible explanations for aggressive social behaviors and nicotine. First, abstinence from nicotine may indirectly result in aggressive social interactions because of the effects of nicotine abstinence on irritability. Alternatively, abstinence from nicotine may directly modify social interactions with others, including increased aggression. Conversely, nicotine administration may decrease irritability in smokers and indirectly reduce aggressive behaviors of smokers. Increased aggressive behaviors during abstinence may, therefore, reflect a change compared with pre-abstinence, non-aggressive behaviors. Similarly, nicotine administration may directly reduce aggressive social interactions. Therefore, increased aggressive behaviors post-abstinence may reflect a change from pre-abstinence, non-aggressive behaviors. The present experiment was conducted to investigate the two direct social interaction possibilities, i.e., whether nicotine administration or cessation directly affected aggressive social interactions.

Sex differences have been reported in the use and effects of nicotine (Grunberg, Winders, & Wewers, 1991). For example, men report that they smoke primarily to reduce stress, and women report that they smoke primarily to control body weight gain (USDHHS, 1988). Men and women also may differ in

social and aggressive behaviors they exhibit while smoking and attempting to quit. Therefore, it is important to include both sexes in a study examining nicotine's effects on social and aggressive behaviors.

The present experiment examined effects of nicotine administration and nicotine cessation on social and aggressive behaviors of male and female Long-Evans rats observed in social interactions with a conspecific. Because social and aggressive behaviors may be affected by different environmental conditions, this experiement included two different housing conditions. In addition, serum testosterone was measured in male rats at the end of the study. The use of an animal model affords a number of advantages to the present study. First, it can be quickly designed and implemented, allowing economy of time and expense. Second, the use of animals allows maximum experimental control over nicotine administration and cessation, and housing conditions. Third, changes in characteristic social and aggressive behaviors exhibited by animals can be used as indices of social and aggressive behaviors during nicotine administration and nicotine cessation. Fourth, the results using an animal model can be used to derive hypotheses for future studies using human populations.

OVERVIEW

The present experiment was designed to examine the effects of nicotine administration, nicotine cessation, and two different housing conditions (individual vs. grouped) on social interactions in rats. In addition, body weights of all subjects were obtained to verify nicotine administration, and serum testosterone was assayed from samples obtained from male rats. Subjects received nicotine (12 mg/kg/day) or saline vehicle for 10 days via osmotic minipump. On the tenth day of nicotine or saline administration, half of the subjects were observed for social interaction behaviors and then sacrificed on the twelfth day of nicotine or saline administration. The other half of the subjects also received either nicotine (12 mg/kg/day) or saline but had minipumps explanted after 14 days administration for observation of social interactions following four days of nicotine or saline cessation. These animals were sacrificed on the fifth cessation day. Behavior observations were conducted on same-sex dyads of comparable body weights from similar housing, drug, and drug administration or cessation conditions. Each dyad was observed for 10 minutes, and social interactions were conducted only once with each dyad to avoid practice effects or a conditioned or learned effect. A total of 16 behaviors were measured during each observation period. Changes in levels of aggressive behaviors exhibited during administration and cessation phases were examined in analyses. Other behaviors also were examined to determine overall effects of nicotine administration, cessation, and differential housing.

HYPOTHESES

There were four major hypotheses and three minor hypotheses. The major hypotheses addressed the effects of nicotine on aggressive behaviors, exploratory and social behaviors, and serum testosterone of male animals. The minor hypotheses were formulated as replications of previous work on gender and housing differences in aggressive behaviors, and the effects of nicotine on body weight.

Major Hypothesis 1: It was hypothesized that nicotine administration would decrease aggressive behaviors. Cessation of nicotine would increase or weaken the decreased aggressive behaviors.

Rationale: Smokers administer aggressive responses under controlled laboratory conditions in an inverse dose-dependent relationship with nicotine (Schechter & Rand, 1974; Cherek, 1981; Cherek et al., 1991). Rats administered acute injections of nicotine display reduced aggressive behaviors compared to those injected with saline (Silverman, 1981). Therefore, it was hypothesized that nicotine administration would decrease aggressive behaviors, and nicotine cessation would increase or attenuate the decreased aggressive behaviors.

Major Hypothesis 2: It was hypothesized that nicotine administration would increase exploratory and social behaviors, and cessation of nicotine would decrease or attenuate the increased social and exploratory behaviors.

Rationale: Nicotine administration increases activity overall for rats measured in a locomotor paradigm (e.g., Grunberg, 1982; Grunberg & Bowen,

1985). Therefore, it was posited that nicotine would increase social and exploratory behaviors of rats measured in a social interaction paradigm.

Nicotine cessation results in inconsistent effects upon activity (Hughes, Higgins, & Hatsukami, 1990). Smoking cessation in humans results in withdrawal symptoms such as increased irritability and depression. Based on these reports, it was hypothesized that nicotine cessation would decrease or attenuate the increased social and exploratory behaviors of rats measured for social interactions.

Major Hypothesis 3: It was hypothesized that nicotine administration would reduce testosterone levels of male rats, and nicotine cessation would increase or attenuate the decreased testosterone levels of male rats.

Rationale: No previous studies have examined the direct effects of nicotine administration or cessation on testosterone levels in rats. Heavy cigarette smoke decreased serum testosterone of male beagles (Mittler, Pogach, & Ertel, 1983).

Major Hypothesis 4: It was hypothesized that the amount of aggressive behaviors displayed by male animals would be directly related to testosterone levels of male animals.

Rationale: Previous research indicates that inter-male aggression or social aggression in male rats is directly related to serum testosterone levels (Albert, Jonik, Watson, Gorzalka, & Walsh, 1990; Albert, Walsh, Gorzalka, Siemens, & Louie, 1986; Albert, Jonik, & Walsh, 1992).

Minor Hypothesis 1: It was hypothesized that males would exhibit more aggressive behaviors than female animals.

Rationale: Research on aggressive behaviors in rats indicates that males exhibit aggressive behaviors under more conditions than female rats (Albert, Jonik, & Walsh, 1992).

Minor Hypothesis 2: It was hypothesized that animals in group-housed conditions would exhibit decreased aggressive behaviors, and decreased social and exploratory behaviors compared to animals in single-housed conditions.

Rationale: Previous research indicates that animals in group or crowded conditions exhibit decreased aggressive behaviors (e.g., Hull, Kastaniotis, L'Hommendieu, & Franz, 1976), and decreased social and exploratory behaviors compared to animals in single-housed conditions (Latané, Cappell, & Joy, 1970; Meaney & Stewart, 1979).

Minor Hypothesis 3: It was hypothesized that nicotine administration would decrease body weight of male and female rats and nicotine cessation would result in a greater rate of weight gain. In addition, these effects would be greater in female than in male animals.

Rationale: Previous nicotine research has demonstrated the inverse relationship between nicotine and body weight, with nicotine exhibiting a greater effect upon body weight in females than males (Grunberg, 1982; Grunberg, 1992; Winders & Grunberg, 1989).

METHODS

Subjects

Subjects were 96 male and 96 female Long-Evans hooded rats, obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in standard polypropylene shoebox cages (42 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri) prior to experimental phases. Animals had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water during all phases of the study. Housing rooms were maintained at 23° C at 50% relative humidity on a 12-hour light/dark cycle (lights on at 1200 hours). At the beginning of the experiment, subjects were approximately 60 days old and weighed approximately 300 g (males) and 200 g (females).

Drug Administration and Surgical Procedure

Nicotine (12 mg/kg/day) or physiologic saline was administered using Alzet osmotic mini-pumps (Model 2002, Alza Corp., Palo Alto, CA). Physiological saline also was used as vehicle for the nicotine solution. Nicotine solution was made from nicotine dihydrochloride. The concentration of 12 mg/kg/day is expressed as nicotine base. Minipumps administered nicotine or saline solution at a rate of approximately 0.47 μl per hour. Dosages were calculated based on body weight such that nicotine animals received 12 mg/kg/day. This method of drug administration was chosen because it avoids the repeated stress of daily injections, and has produced results in rats that have been replicated in studies of human smokers. Similarly, this drug dose has produced behavioral effects in rats that approximate those in human smokers (Grunberg, 1992; Winders &

Grunberg, 1989).

Subjects were anesthetized by placing them individually in a bell jar containing a sterile gauze dampened with methoxyfluorane (Metophane) as the anesthetic, and a wire mesh barrier separating each subject from the anesthesia source. When the animal showed lack of response to a tail-pinch test (duration of exposure to anesthesia approximately 60-90 seconds), the animal was removed and a 3 x 5 cm area between the animal's withers was shaved and cleaned with an iodine-based antiseptic (Betadine). A 2 cm horizontal incision was made with blunt-nosed surgical scissors, a subcutaneous pocket was created by spreading the subcutaneous tissues with the scissor tips, and a mini-pump was inserted with the flow modulator oriented towards each subject's head. The incision was closed with 9 mm stainless steel wound clips. The entire surgical procedure took approximately 4 minutes.

Withdrawal Phase subjects also had minipumps explanted after 14 days of nicotine or saline administration. Before explanting the minipumps, anesthesia was administered as described above. A 2.5 x 4 cm area surrounding the implanted minipump was shaved and cleaned with Betadine. A 1.5 cm incision was made at the base of the implanted minipump and the minipump was removed. The incision was closed with 9 mm stainless steel wound clips. The entire surgical procedure took approximately 3 minutes.

Environmental Manipulation

During the Baseline Phase all subjects were individually housed in standard shoebox cages. At the beginning of the Experimental Phase, subjects were assigned to one of two different housing conditions based on Brown & Grunberg

(1995), either individual housing or a group of six same-sex subjects per cage. The environmental manipulation was done twenty-four hours after surgical implantation of minipumps. The individually-housed subjects were transferred to clean standard shoebox cages. The group-housed subjects were placed in clean standard shoebox cages and floor space per animal was adjusted with a plexiglass divider to establish approximately 55% of U.S. Department of Health and Human Services (USDHHS) recommended floor space per animal. USDHHS floor space recommendations are based on body weight ranges. Because of different mean body weights, males and females required cages with different amounts of floor space. Grouped males were placed in standard shoebox cages (six subjects per cage). This cage size provided approximately 143.5 cm^2 of floor space per male subject (55% of DHHS recommended floor space for weight range 300-400 g). Grouped females were placed in standard shoebox cages (six subjects per cage) but the amount of floor space was adjusted using a polypropylene divider bolted to the cage top. The divider was placed so that each female subject had approximately 102.9 cm^2 of floor space (55% of DHHS recommended floor space for weight range 200-300 g). Individually-housed animals had cages changed twice a week. Group-housed subjects' cages were changed every other day and were checked twice daily to insure that subjects had adequate food and water.

Procedure

Baseline phase. Animals were gentled in pairs for a two-minute period for three consecutive days prior to baseline and experimental phases. Following a seven-day baseline phase, animals were implanted with minipumps and were assigned to differential housing conditions one day following surgery.

Administration phase. On day 10 of nicotine or saline administration, 96 animals of similar sex, housing, and drug conditions were paired and were evaluated for social interaction. These animals were sacrificed on the twelfth day of drug administration to obtain samples for biochemical analyses.

Cessation phase. The 96 cessation-phase animals had minipumps explanted following 14 days of nicotine administration, and then were returned to their grouped or individual housing condition. On the fourth cessation day, these subjects were paired by similar body weights in same sex, housing, and drug condition pairs, and were evaluated for social interaction. These animals were sacrificed on the fifth day of nicotine or saline cessation to obtain samples for biochemical analyses.

Dependent Variables

Body Weight. Animals were weighed throughout the experiment. Body weights were determined by placing each subject in a weighing pan on an electronic balance (Sartorius, Inc., Model MC-1) that was programmed to provide the mean of 10 independent measurements taken at 1 sec intervals.

Social Interaction Evaluation. Social Interaction evaluations were conducted in a 44 x 44 cm clear plexiglass arena, with clear plexiglass walls 30 cm in height. A brown opaque shield measuring 45 cm in height was placed

around three sides of the test arena at a distance of 10 cm. A 100-watt bulb was suspended in a hooded light fixture 40 cm above the floor of the arena. A video camera was placed 30 cm away from the unshielded side of the arena for videotaping of animal behaviors. Video cable connected cameras to taping equipment in another procedure room adjacent to the testing room.

Following each social interaction, arenas were cleaned with a commercial alcohol-based cleaning agent (Fantastic) and were wiped dry. Cardboard shields and lighting were then repositioned for the next interaction.

Animals were paired for testing with another same-sex animal in the same housing and drug condition. Pairs were assigned to insure comparable body weights. To evaluate social interactions, animals were placed in opposite corners of the arena and a slotted plexiglass lid was placed on top of the arena. Animal social interactions were videotaped for ten minutes for later scoring and analyses of animal behaviors.

Videotapes made during the testing sessions were scored by a sampling procedure in which the behavior of each animal was scored once every three seconds for the entire 10 min period. Behaviors were separated into three major categories for scoring: exploratory, social, and other. (Appendix I, II, and III include a copy of the scoring sheet, the scoring protocol used by raters, and the behavior scores summary form.) Two raters were trained by the author to score videotapes, and an inter-rater reliability score was computed for each rater after training. This score was determined by correlation comparison of the overall totals of each behavior that each rater scored for the same animal in one ten-minute social interaction. Each rater was trained until they attained at least a

0.90 correlation with the scoring of the author. The raters' scorings also were checked at every tenth animal they scored to ensure that their inter-rater reliability continued to be at least 0.90 or greater. In one instance when one rater's scoring dropped below this mark, the author re-scored the ten interactions previous scored by that rater, and the re-trained the rater until he was again above the 0.90 mark. Behaviors were scored according to the following guidelines:

Exploratory behaviors were scored considering five different types.

Freeze. The animal stops all movement, including total movement of the head.

Sniff. The animal has stopped locomotion but continues to sniff the area around it. The animal may move its head and sniffing motions, such as motion around the nostrils and whiskers, are discernible.

Move. The animal moves but does not have its head down and is not engaged in sniffing activities.

Move + Sniff. The animal moves and has its head down and is actively sniffing the area.

Rear. The animal stops moving on four feet and shifts its weight back to its hindfeet. Typically, the animal rises up to investigate the sides of the test arena, but also may investigate the other animal.

Social behaviors considered five types.

Touch. The animal is in physical contact with the other animal (e.g., touching tails, brushing hind-quarters).

Follow. The animal moves in the same path following the other animal.

Sniff Other. The animal sniffs the other animal.

Groom other. The animal engages in discernible grooming activity of the other animal.

Wrestle. The animal engages in wrestling activity with the other animal. Wrestling involves pushing with the front or back paws or upper body against the other animal.

The remaining category, entitled *other*, consisted of the following types of social interaction behaviors:

Groom self. The animal squats on hind legs and grooms itself with front paws.

Box/Bite. Boxing occurs when two animals are reared up on hind legs facing each other and attempt to push each other with their front paws. Biting typically occurs when an animal moves around the side of another animal and bites it on the back of the neck. One category was used to score these two behaviors for ease in scoring.

Eat. The animal can be seen eating something. Because no food is present in the test arena, the item eaten is usually feces.

Submissive/On Top. The rat assumes a submissive posture (i.e., flat on back, paws up). The other animal is on top. One category was used to score these two behaviors for ease in scoring.

Other. This residual category was used to score behaviors not captured in categories above. An example of a behavior that was scored in this category included two occasions in which an animal was observed momentarily pulling on the surgical staples of the other animal with its teeth (No injury occurred to either animal as a result of this behavior).

Testosterone. At the end of the drug administration phase as well as at the end of cessation phase, half of the subjects were sacrificed by decapitation without anesthesia. Trunk blood was collected into 13 ml polypropylene tubes in an ice bucket. Samples were centrifuged at 3000 rpm (1500 g), 4° C in a tabletop refrigerated centrifuge (IEC Centra, Model GP8R). Serum was removed from each tube using a plastic pipette and pre-specified quantities of serum were transferred to several sets of screw-cap Eppindorf tubes for later biochemical assays. Samples were stored at -80° C until removed to conduct assay procedures. One set of Eppindorf tubes was used for testosterone assays of the male subjects only. Other sets were saved for other biochemical assays not reported here.

Serum testosterone was measured by a standard, double-antibody radioimmunoassay (Equate® RIA, Binax, Portland, ME). This assay has accuracy of at least 92%, precision of at least 92%, and sensitivity of at least 95% in the range ≤ 15 ng/dl. The radioactive counts were converted to concentration (ng/ml) values by Spline function analysis of the standard curve.

Data analyses

Body Weight. Body weight data were analyzed by an overall three-way repeated measures analysis of covariance (ANCOVA) covarying for mean baseline body weights of each sex. All analyses used time as the within-subject factor, and drug and housing condition as between-subjects factors. Separate analyses were conducted for males and females during administration and during cessation phases. Males and females were analyzed separately because significant body weight differences existed between the sexes at all time points.

In addition, the empirical literature indicates that effects of nicotine on body weight are greater in females than in males. Separate analyses were conducted of during-administration phase subjects (hereafter denoted as During-phase) and during-cessation phase subjects (hereafter denoted as Cessation-phase) in order to include body weight data collected during cessation phase in analyses of Cessation-Phase subjects. Five time points were used for analyses of During-phase male and female subjects: Day 4 of baseline, and Days 1, 6, 11, and 13 during drug administration. Analyses of body weights for Cessation-phase subjects included Day 4 of baseline, Days 1, 6, 13, and 15 during drug administration, and Day 3 of nicotine or saline cessation for a total of six time points. Separate ANCOVAs with baseline body weight as covariate and factors of drug and housing condition were conducted at each time point to determine which groups differed significantly. Statistical significance was based on two-tailed distributions with an alpha level of 0.05.

Social Interaction Evaluation. Because social interaction behaviors are interdependent, dyad scores were computed for purposes of all statistical analyses. Dyad scores simply were sums of the values for the two subjects of each dyad for each of the individual behaviors listed above. Appendix II presents a sample score sheet used to compute dyad scores. Social interaction dyad data were analyzed first by creating a matrix of all animal behaviors measured to determine associations of scoring behavior clusters for During-phase and Cessation-phase animals. Behaviors that correlated significantly and met criteria for parametric tests were analyzed using multivariate analysis of variance (MANOVA) tests. Behaviors that did not correlate significantly were

analyzed using separate analysis of variance (ANOVA) tests. Behaviors that did not meet parametric data criteria, aggressive behaviors and fecal boli data, were analyzed using Kruskal-Wallis non-parametric tests. Statistical significance was based on two-tailed distributions with an alpha level of 0.05. Separate analyses were conducted for males and females during administration and during cessation phases.

For aggressive behaviors, total dyad scores of wrestling and bite/box behaviors were summed to obtain an overall aggression score for each dyad. These behaviors adequately correlated ($r = 0.54$, $p < 0.01$). Because aggressive behavior data did not meet criteria for parametric tests, aggression scores were analyzed by Kruskal-Wallace tests to examine hypothesized differences between sex, housing, phase, and drug conditions. In addition, multiple regression-correlation analyses were conducted to determine what proportion of the variance in aggressive behaviors was accounted for by drug, sex, housing, and testosterone variables.

Testosterone. Testosterone data were analyzed by a three-way between-subjects analysis of variance, with drug, sex, and housing inserted as variables. Subsequent univariate ANOVAs were performed. Statistical significance was based on two-tailed distributions with an alpha level of 0.05. In addition, Pearson product-moment correlations were computed between the dyad testosterone values and aggression scores, and between individual testosterone and individual bite/box and wrestle scores.

RESULTS

Body Weight

Drug effects, During-phase subjects. Figures 1 through 4 present body weights in grams of male and female animals for administration phase and cessation phase subjects at multiple time points. Nicotine decreased body weight gains over time for males [F (4, 172) = 37.274, $p < 0.05$] and females [F (4, 172) = 9.912, $p < 0.05$].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine-treated males weighed less than saline-treated males [F (1, 43) = 29.167, $p < 0.05$], and nicotine-treated females weighed less than saline-treated females [F (1, 43) = 26.180, $p < 0.05$]. These effects for males were significant on Day 6 [F (1, 43) = 28.071, $p < 0.05$], Day 11 [F (1, 43) = 37.294, $p < 0.05$], and Day 13 [F (1, 43) = 49.941, $p < 0.05$]. These effects for females were significant on Day 6 [F (1, 43) = 24.094, $p < 0.05$], Day 11 [F (1, 43) = 30.554, $p < 0.05$], and Day 13 [F (1, 43) = 19.263, $p < 0.05$]. In addition, nicotine reducing body weight of group-housed females more than single-housed females on Day 11 [F (1, 43) = 4.172, $p < 0.05$].

Drug effects, Cessation-phase subjects. Body weights of nicotine-cessation animals increased more over time than did body weights of saline-cessation animals. This effect was revealed for both males [F (5, 210) = 17.488, $p < 0.05$] and for females [F (5, 215) = 24.782, $p < 0.05$].

Follow-up analyses for Drug effects, Cessation-phase subjects. Nicotine-cessation males weighed less than saline-cessation males [F (1, 42) = 15.321, p

< 0.05]. The same finding held for females [F (1, 43) = 58.011, p < 0.05]. These effects for males were significant on Day 6 [F (1, 43) = 18.304, p < 0.05], Day 13 [F (1, 43) = 23.726, p < 0.05], Day 15 [F (1, 42) = 32.328, p < 0.05], and cessation Day 3 [F (1, 43) = 7.925, p < 0.05]. These effects for females were significant on Day 6 [F (1, 43) = 61.881, p < 0.05], Day 13 [F (1, 43) = 52.325, p < 0.05], Day 15 [F (1, 43) = 82.147, p < 0.05] and cessation Day 3 [F (1, 43) = 27.193, p < 0.05].

Housing effects, During-phase subjects. Group-housing decreased body-weights over time for both males and females compared to single-housed males [F (4, 172) = 3.287, p < 0.05] and females [F (4, 172) = 2.989, p < 0.05].

Follow-up analyses for Housing effects, During-phase subjects. In contrast to the overall finding, group-housed females weighed more than single-housed females at one time-point, on Day 11 [F (1, 43) = 6.747, p < 0.05].

Housing effects, Cessation-phase subjects. Body-weights of group-housed males increased at a slower rate over time than for single-housed males [F (5, 210) = 5.499, p < 0.05]. In addition, there was a trend for the same effect in females [F (5, 215) = 2.220, p = 0.053].

Follow-up analyses for Housing effects, Cessation-phase subjects. Group-housed males weighed less than single-housed males [F (1, 42) = 5.099, p < 0.05]. A trend in the same direction was revealed for Cessation-phase females [F (1, 43) = 3.228, p = 0.079]. Group-housed males weighed less than single-housed males on Day 6 [F (1, 43) = 9.810, p < 0.05], Day 15 [F (1, 42) = 6.102, p < 0.05], and cessation Day 3 [F (1, 43) = 4.795, p < 0.05]. A similar effect was revealed for females on cessation Day 3 [F (1, 43) = 5.368, p < 0.05].

Social Interaction Evaluation

Correlations, During-phase subjects. Table 1 presents the correlation matrix obtained for the observed animal behaviors for During-phase animals. Significant correlations for During-phase animals included Box with Wrestle behaviors (+0.478, $p < 0.01$); Follow with Touch (+0.498, $p < 0.01$); Follow with Sniff-Other (+0.519, $p < 0.01$); Freeze with Bolus (+0.339, $p < 0.05$) and Groom-Self (+0.285, $p < 0.05$); and Touch correlated significantly with Sniff-Other (+0.307; $p < 0.05$).

Correlations, Cessation-phase subjects. Table 2 presents the correlation matrix obtained for the observed animal behaviors for Cessation-phase animals. Significant correlations for Cessation-phase animals included Box with Wrestle behaviors (+0.633, $p < 0.01$); Follow with Touch (+0.354, $p < 0.05$); and Follow correlated significantly with Sniff-Other (+0.669, $p < 0.01$).

Aggressive Behaviors

Drug effects, During-phase subjects. Figure 5 presents mean number of aggressive behaviors per dyad for each treatment group. A summary of the results of analyses conducted on aggressive behavior data is presented in the third column of Table 4. Nicotine-treated animals exhibited fewer aggressive behaviors than saline-treated animals (Kruskal-Wallis [KW] $\chi^2 = 5.695$, $df = 1$, $p < 0.05$).

Follow-up analyses for Drug effects, During-phase subjects. Nicotine somewhat reduced aggressive behaviors of females overall ($\chi^2 = 3.324$, $df = 1$, $p = 0.068$), single-housed males ($\chi^2 = 2.847$, $df = 1$, $p = 0.092$), and single-housed females ($\chi^2 = 3.718$, $df = 1$, $p = 0.054$) compared to saline-treated animals.

Drug effects, Cessation-phase subjects. No significant effects for Drug on aggressive behaviors were noted for Cessation-phase animals.

Housing effects, During-phase subjects. Group-housed animals exhibited fewer aggressive behaviors overall than single-housed animals ($\chi^2 = 12.967$, $df = 1$, $p < 0.01$).

Follow-up analyses for Housing effects, During-phase subjects. Group-housed males and group-housed females exhibited less aggression than single-housed males ($\chi^2 = 7.182$, $df = 1$, $p < 0.01$) and single-housed females ($\chi^2 = 5.909$, $df = 1$, $p < 0.05$).

Housing effect, Cessation-phase subjects. Group-housing somewhat reduced aggressive behaviors of Cessation-phase animals overall ($\chi^2 = 2.838$, $df = 1$, $p = 0.092$).

Follow-up analyses for Housing effect, Cessation-phase subjects. No additional significant effects for housing on aggressive behaviors were noted for Cessation-phase animals.

Multiple Regression/Correlation analyses of Aggressive behaviors. Multiple regression/correlation analyses indicated that drug, sex, and housing accounted for 24% of the variance in aggressive behaviors exhibited by animals in During-administration phase social interactions, and the contributions of drug and housing both were significant ($p < 0.05$). For males, housing and drug accounted for 27% of the variance in aggressive behaviors, and housing was a significant predictor ($p < 0.05$). Drug was a significant predictor of aggressive behaviors in single-housed male animals, accounting for 33% of the variance ($p < 0.05$). For females, housing and drug accounted for 16% of the variance in

aggressive behaviors, and the contribution of drug approached significance ($p < 0.10$). Separate MRC analyses of Cessation-phase animals revealed no significant predictors of aggressive behaviors for these subjects.

Social Behaviors--Touch

Drug effects, During-phase subjects. Figure 6 presents number of touch behaviors per dyad for each treatment group. The results of analyses conducted on touch behavior data are summarized in the first column of Table 3. Nicotine somewhat reduced touching behaviors of group-housed animals [F (1, 40) = 3.474, $p = 0.07$].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine reduced touching behaviors of group-housed females, whereas single-housed females touched equivalent amounts [F (1, 40) = 4.378, $p < 0.05$]. Nicotine reduced touching behaviors of group-housed males and group-housed females compared to saline-treated group-housed males [F (1, 10) = 5.033, $p < 0.05$], and saline-treated group-housed females [F (1, 10) = 6.161, $p < 0.05$].

Drug effects, Cessation-phase subjects. No significant effects for Drug on touching behaviors of Cessation-phase treatment groups were revealed.

Housing effects, During-phase subjects. Group housing reduced touching behaviors of animals [F (1, 40) = 35.262, $p < 0.05$]. Among single-housed animals, males exhibited more touches than females, but group-housed animals touched equivalent amounts [F (1, 40) = 12.636, $p < 0.05$].

Follow-up analyses for Housing effects, During-phase subjects. Group-housing reduced touches of male animals compared to single-housed males [F (1, 20) = 37.197, $p < 0.05$]. This effect also was revealed as a trend for females

[F (1, 20) = 3.601, p = 0.072], in the same direction.

Housing effects, Cessation-phase subjects. No significant effects for Housing on touching behaviors were revealed for Cessation-phase treatment groups.

Social Behaviors--Sniff Other

Drug effects, During-phase subjects. Figure 7 presents mean number of Sniff Other behaviors per dyad for each treatment group. The results of analyses conducted on sniff other behavior data are summarized in the second column of Table 3. Nicotine-treated animals sniffed conspecifics less than saline-treated animals [F (1, 40) = 12.756, $p < 0.05$]. In addition, nicotine reduced sniff-other behaviors of group-housed animals, whereas single-housed animals sniffed conspecifics equivalent amounts [F (1, 40) = 11.590, $p < 0.05$].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine somewhat reduced sniff-other behavior in males compared to saline-treated males [F (1, 20) = 3.970, p = 0.06]. Nicotine reduced sniffing of conspecifics in both group-housed males and group-housed females compared to saline-treated group-housed males [F (1, 10) = 11.880, $p < 0.05$] and females [F (1, 10) = 11.063, $p < 0.05$].

Drug effects, Cessation-phase subjects. Nicotine-cessation animals sniffed conspecifics less than saline-cessation animals [F (1, 40) = 3.743, $p < 0.05$].

Follow-up analyses for Drug effects, Cessation-phase subjects. Nicotine-cessation females sniffed conspecifics less than saline-cessation females [F (1, 20) = 6.844, $p < 0.05$]. Nicotine-cessation group-housed females sniffed conspecifics less that saline-cessation group-housed females [F (1, 10) =

19.483, $p < 0.05$].

Housing effects, During-phase subjects. Group-housed subjects sniffed conspecifics less than single-housed subjects [F (1, 40) = 27.922, $p < 0.05$].

Follow-up analyses for Housing effects, During-phase subjects. Group-housed males and females sniffed conspecifics less than single-housed males [F (1, 20) = 6.092, $p < 0.05$] and females [F (1, 20) = 24.879, $p < 0.05$].

Housing effects, Cessation-phase subjects. A trend toward a significant main effect for Housing was revealed for Cessation-phase animals, in the same direction [F (1, 40) = 3.743, $p = 0.06$].

Follow-up analyses for Housing effects, Cessation-phase subjects. Group-housed females sniffed conspecifics less than single-housed animals [F (1, 20) = 6.844, $p < 0.05$].

Social behaviors--Follow

Main analysis for Drug effects, During-phase subjects. Figure 8 presents mean number of following behaviors per dyad for each treatment group. The results of analyses conducted on follow behavior data are summarized in the third column of Table 3. Analyses for each phase and sex found neither significant main effects for Drug for this social behavior, nor significant interactions including drug as an interacting variable.

Housing effects, During- and Cessation-phase subjects. Group-housed animals exhibited less following behavior than single-housed animals in During-phase [F (1, 40) = 10.435, $p < 0.05$] and Cessation-phase [F (1, 40) = 20.074, $p < 0.05$] social interactions. Group-housing reduced following compared to single-housed animals for During-phase males [F (1, 20) = 8.871, $p < 0.05$], Cessation-

phase males [F (1, 20) = 12.346, $p < 0.05$], and Cessation-phase females [F (1, 20) = 7.777, $p < 0.05$].

Other Behaviors--Groom Self

Drug effects, During-phase subjects. Figure 9 presents mean number of grooming behaviors per dyad for each treatment group. The results of analyses conducted on groom self behavior data are summarized in the second column of Table 4. Nicotine-treated animals groomed more than saline-treated animals [F (1, 40) = 6.219, $p < 0.05$].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine increased grooming behaviors of group-housed females, but single-housed females exhibited equivalent amounts of grooming behavior regardless of drug treatment [F (1, 20) = 4.773, $p < 0.05$].

Housing effects, During-phase subjects. Group-housed animals groomed more than single-housed animals [F (1, 40) = 36.677, $p < 0.05$].

Follow-up analyses for Housing effects, During-phase subjects. Group-housed males and females exhibited more grooming behaviors than single-housed males [F (1, 20) = 19.642, $p < 0.05$] and females [F (1, 20) = 21.166, $p < 0.05$].

Housing effects, Cessation-phase subjects. Group-housed animals groomed more than single-housed animals [F (1, 40) = 29.025, $p < 0.05$].

Follow-up analyses for Housing effects, Cessation-phase subjects. Group-housed males and females grooming more than single-housed males [F (1, 20) = 13.721, $p < 0.05$] and females [F (1, 20) = 19.642, $p < 0.05$].

Other behavior--Bolus

Drug effects, During- and Cessation-phase subjects. Figure 10 presents mean number of boluses counted per dyad for each treatment group. The results of analyses conducted on bolus behavior data are summarized in the first column of Table 4. Kruskal-Wallis non-parametric tests did not reveal significant main effects or interactions with Drug for each phase or sex.

Housing effects, During-phase subjects. Group-housed animals deposited more boluses during social interactions than single-housed animals [$\chi^2 = 4.150$, $df = 1$, $p < 0.05$].

Housing effects, Cessation-phase subjects. No significant main effects for Housing upon boluses deposited in social interaction tests were revealed for Cessation-phase animals.

Exploratory Behaviors--Freeze

Drug effects, During-phase subjects. Figure 11 presents mean number of freezing behaviors per dyad for each treatment group. A summary of results of analyses conducted on freezing behavior data is presented in the first column of Table 5. Nicotine-treated animals engaged in more freezing behaviors than saline-treated animals [F (1, 40) = 10.681, $p < 0.05$].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine-treated males froze more than saline-treated males [F (1,20) = 10.104, $p < 0.05$]. A trend in the same direction was revealed for females [F (1, 20) = 3.862, $p = 0.063$]. Nicotine-treated group-housed males froze more than saline-treated group-housed males [F (1, 10) = 6.036, $p < 0.05$]. This effect was also revealed as a trend for single-housed males [F (1, 10) = 4.44, $p = 0.061$], and group-

housed females [F (1, 10) = 3.914, p = 0.076], in the same direction.

Drug effects, Cessation-phase subjects. No significant effects for Drug on freezing behaviors were revealed for Cessation-phase subjects.

Housing effects, During-phase subjects. Group-housed animals exhibited more freezing behaviors than single-housed animals [F (1, 40) = 6.383, p < 0.05].

Follow-up analyses for Housing effects, During-phase subjects. Group-housed females exhibited greater amounts of freezing behavior than single-housed females [F (1, 20) = 4.045, p = 0.058].

Housing effects, Cessation-phase subjects. No significant effects for housing upon freezing behavior were noted for Cessation-phase animals.

Exploratory Behaviors--Move

Drug effects, During-phase subjects. Figure 12 presents mean number of move behaviors per dyad for each treatment group. A summary of the results of analyses conducted on moving behavior data is presented in the second column of Table 5. Nicotine-treated animals moved more than saline-treated animals in During-phase social interactions [F (1, 40) = 10.722, p < 0.05]. In addition, there was a trend for nicotine to increase movement of group-housed animals, whereas single-housed animals moved equivalent amounts [F (1, 40) = 3.651, p = 0.063].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine-treated males and females moved more than saline-treated males [F (1, 20) = 4.419, p < 0.05] and females [F (1, 20) = 9.357, p < 0.05]. Nicotine increased movement of group-housed females compared to saline-treated females, but

single-housed females moving equivalent amounts regardless of nicotine or saline treatment [F (1, 20) = 4.677, $p < 0.05$]. Nicotine-treated group-housed females moved more than saline-treated group-housed females [F (1, 10) = 12.083 $p < 0.05$]. This effect also was revealed as a trend for group-housed males [F (1, 10) = 3.526, $p = 0.09$], in the same direction.

Drug effects, Cessation-phase subjects. No significant effects for drug on animal movement in social interactions were noted for Cessation-phase animals.

Housing effects, During-phase subjects. Single-housed males moved more than single-housed females, but group-housed animals moved equivalent amounts regardless of sex [F (1, 40) = 7.784, $p < 0.05$].

Follow-up analyses for Housing effects, During-phase subjects. In males, there was a trend for group housing to decrease movement compared to single housing [F (1, 20) = 3.610, $p = 0.072$]. In contrast, group housing increased movement for females compared to single-housed females [F (1, 20) = 5.743, $p < 0.05$].

Housing effects, Cessation-phase subjects. Single-housed animals moved more in social interactions than group-housed animals [F (1, 40) = 5.608, $p < 0.05$]. In addition, single-housed males moved more than single-housed females, but group-housed animals moved equivalent amounts regardless of sex [F (1, 40) = 4.962, $p < 0.05$].

Follow-up analyses for Housing effects, Cessation-phase subjects. Single-housed males moved more than group-housed males [F (1, 20) = 11.869, $p < 0.05$].

Exploratory Behaviors--Rear

Drug effects, During-phase subjects. Figure 13 presents mean number of rearing behaviors per dyad for each treatment group. A summary of analyses conducted on rearing behavior data is presented in the third column of Table 5. Nicotine-treated animals exhibited fewer rearing behaviors than saline-treated animals [F (1, 40) = 12.165, $p < 0.05$]. There was a trend for nicotine to reduce rearing of group-housed animals, but single-housed animals exhibited equivalent amounts of rearing regardless of drug treatment [F (1, 40) = 3.662, $p = 0.063$].

Follow-up analyses for Drug effects, During-phase subjects. Nicotine reduced rearing behaviors of males and females compared to saline-treated males [F (1, 20) = 4.268, $p < 0.05$] and saline-treated females [F (1, 20) = 8.436, $p < 0.05$]. There was a trend for nicotine to decrease rearing behavior of group-housed females, but single-housed females reared equivalent amounts regardless of drug treatment [F (1,20) = 3.904, $p = 0.062$]. Nicotine reduced rearing behavior of group-housed females compared to saline-treated females [F (1, 10) = 8.787, $p < 0.05$]. There was a trend in the same direction for group-housed males [F (1, 10) = 3.667, $p = 0.085$].

Drug effects, Cessation-phase subjects. There was a trend for nicotine-cessation females to rear more than nicotine-cessation males, but saline-cessation animals exhibited equivalent amounts of rearing behaviors [F (1, 40) = 3.019, $p = 0.09$].

Follow-up analyses for Drug effects, Cessation-phase subjects. There was a trend for nicotine-cessation single-housed females to decrease rearing behaviors, whereas group-housed cessation-phase females reared equivalent

amounts [F (1, 20) = 3.157, p = 0.091].

Housing effects, During- and Cessation-phase subjects. No significant main effects for Housing upon rearing behaviors were revealed. Significant interactions with housing are described above.

Testosterone

Drug effects, During-phase subjects. Figure 16 presents mean serum testosterone level per dyad for each treatment group. No significant main effects for Drug were revealed.

Follow-up analyses for Drug effects, During-phase subjects. To determine if any effects for Drug on testosterone levels existed as hypothesized, subsequent analyses were conducted. Nicotine administration reducing testosterone of single-housed males, but did not have this effect in group-housed males [F (1, 17) = 3.88, p = 0.065]. MRC analyses revealed that male testosterone levels accounted for less than 1% of the variance in aggressive behaviors of male animals.

CONFIRMATION OF HYPOTHESES

Major Hypothesis 1: The hypothesis that nicotine administration would decrease aggressive behaviors and cessation of nicotine would increase or attenuate the decreased aggressive behaviors was partially confirmed. Overall, nicotine administration decreased aggressive behaviors. Cessation of nicotine attenuated the decreased aggressive behaviors in single-housed males.

Major Hypothesis 2: The hypothesis that nicotine administration would increase exploratory and social behaviors, and cessation of nicotine would decrease or attenuate the increased social and exploratory behaviors was partially confirmed. For exploratory behaviors, nicotine administration increased moving and freezing behaviors, but decreased rearing behaviors. Nicotine cessation attenuated these effects. For social behaviors, nicotine administration decreased sniffing of conspecifics but had no effect on touching or following behaviors. These effects also were observed during nicotine or saline cessation, with nicotine cessation animals sniffing conspecifics less than saline cessation animals.

Major Hypothesis 3: The hypothesis that nicotine administration would reduce serum testosterone levels of male rats, and nicotine cessation would increase or weaken the decreased testosterone levels of male rats was partially confirmed. Nicotine reduced serum testosterone of male rats as hypothesized, but this effect was revealed only in single-housed males. Nicotine cessation attenuated the decreased testosterone levels of single-housed male rats as hypothesized.

Major Hypothesis 4: The hypothesis that the amount of aggressive behaviors displayed by male animals would be directly related to testosterone levels of male animals was disconfirmed. MRC analyses revealed that male testosterone levels accounted for less than 1% of the variance in aggressive behaviors of male animals.

Minor Hypothesis 1: The hypothesis that males would exhibit more aggressive behaviors than female animals was disconfirmed. There were no significant differences in aggressive behaviors exhibited by male and female subjects. Overall, effects of nicotine administration and cessation on aggressive behaviors were similar in male and female rats.

Minor Hypothesis 2: The hypothesis that animals in group-housed conditions would exhibit decreased aggressive behaviors and decreased social and exploratory behaviors compared to animals in single-housed conditions was confirmed.

Minor Hypothesis 3: The hypothesis that nicotine administration would decrease body weight of male and female rats was confirmed.

DISCUSSION

The present experiment examined the effects of nicotine administration, nicotine cessation, and two different housing conditions (individual vs. grouped) on social interactions and serum testosterone in rats. It was hypothesized that nicotine administration would decrease aggressive behaviors and testosterone and that nicotine cessation would increase aggressive behaviors and testosterone. It also was hypothesized that group housing would decrease aggressive behaviors.

Nicotine decreased body weights of subjects, validating administration of nicotine and replicating previous research reports (Grunberg, 1992; Winders & Grunberg, 1989). As hypothesized, nicotine administration reduced aggressive behaviors and housing affected these behaviors. Overall, nicotine reduced aggressive behaviors of male and female rats, but these effects were a result of nicotine in the single-housed conditions. Effects of nicotine on serum testosterone in males also depended on housing condition with nicotine reducing serum testosterone in single-housed males, but not in group-housed males. However, these effects of nicotine to reduce serum testosterone accounted for less than one percent of the variance in aggressive behaviors in the males. Therefore, despite nicotine's effect to reduce testosterone, this effect did not appear to mediate the effects of nicotine to decrease aggressive behavior. In addition, housing altered aggressive behaviors, with single-housed animals exhibiting more aggressive behaviors than group-housed animals, also replicating previous research reports (e.g., Meaney & Stewart, 1979).

For exploratory behaviors, nicotine administration increased horizontal movement of male and female rats. Therefore, the effect of nicotine to decrease aggressive interactions cannot be attributed simply to animals moving around less and simply knocking into each other less. Nicotine administration also decreased rearing behaviors, and increased freezing behaviors. Nicotine cessation decreased these effects. Differential housing had inconsistent effects upon exploratory behaviors.

For social behaviors, sniffing other animals decreased during nicotine administration as well as during nicotine cessation. Nicotine administration and cessation did not affect following and touching. Housing conditions had a more robust effect upon non-aggressive social behaviors than did nicotine administration, with group-housed animals displaying less following, touching, and sniff-other behaviors than single-housed animals, replicating previous research reports (e.g., Latané, Cappell, & Joy, 1976; Meaney & Stewart, 1979).

For other non-aggressive behaviors, nicotine administration increased grooming of male and female animals, but had no effect on fecal boli deposited during social interactions. Nicotine cessation did not reveal any effects on these behaviors. Housing conditions had a clear effect on grooming behaviors, with group-housing increasing grooming behaviors compared to single-housed animals. Group-housed animals also deposited more fecal boli during social interactions than did single-housed animals.

Taken together, the results suggest that the effects of nicotine may be modified by environmental or social situations. Specifically, the effects of nicotine on aggressive behaviors (as well as on other behaviors) was influenced

by housing conditions. Further, for male subjects, aggressive behaviors were accounted for by housing and drug conditions rather than by testosterone levels. Changes in the effects of nicotine because of environmental conditions may contribute to changes in smoking behaviors and behaviors of smokers. For example, if the present results replicate with humans, then it would suggest that smokers who live in grouped or crowded conditions may not experience the effects of nicotine to reduce aggressive behaviors. In contrast, smokers who live alone or under conditions of substantial open space may experience a marked decrease in aggressive or impulsive behaviors when smoking. More broadly, other environmental factors, such as number of interactions per day, workplace crowding, or job environment, may act like group versus single housing and interact with the effects of nicotine on aggressive behaviors and social interactions. These actions of housing conditions upon nicotine's effects may result in the increase in smoking behaviors observed in the subgroup of smokers who smoke very little during the day, but smoke more at home (e.g., "peak-seekers").

Moreover, the present findings suggest that some smokers may smoke to reduce aggressive or impulsive behaviors. Perhaps some smokers are aware of the effects of nicotine to reduce aggressive behaviors and, therefore, smoke as a form of impulse control or self-medication. If this self-medication is the case, then it may be a desired effect of nicotine and smoking that contributes to smoking maintenance and dissuades smokers from abstaining. Further, if smoking is being used for impulse control, then smoking cessation programs should include (for some smokers) impulse control techniques to help smokers to

successfully abstain as well as to avoid relapse. The possible extensions of the present findings deserve research attention. A study in a sample of human smokers prone to aggressive behaviors, such as a sample of selected, aggressive prisoners who were seeking assistance with smoking cessation, may be useful to determine if the results of the present study generalize to human smokers.

It is noteworthy that there are several limitations of the present experiment. First, nicotine dependence or withdrawal phenomena were not evaluated in the present experiment. Instead, the present experiment used a well-established animal paradigm that has yielded behavioral results that parallel human findings (Grunberg, 1982) and that has yielded dependency responses in other studies (Malin, Lake, Newlin-Maultsby, Roberts, Lanier, et al., 1992). To more closely parallel human smokers, future studies could extend the period of nicotine administration. In addition, future studies could be conducted using a strain of rats selectively bred to exhibit higher levels of aggression (R. Blanchard, personal communication, 1995) and using experimental conditions to initiate aggression, such as cohabitation with female rats and sexual experience (Albert et al., 1992), to more precisely determine if withdrawal following nicotine cessation results in an increase in aggressive behaviors in social interactions. Also, the present experiment could be repeated with nicotine self-administration paradigms in rats (Corrigall & Coen, 1989; Cox, Goldstein, & Nelson, 1984).

Experiments examining the effects of nicotine abstinence or smoking cessation on aggressive behaviors have relevance for health-care professionals planning tobacco cessation interventions in large group settings as part of

preventive health programs of health maintenance organizations. One relevant example is the U.S. military, which has a reported higher prevalence of smokers than in the civilian population, and has declared an objective of dramatically reducing smoking prevalence by the year 2000 (Kroutil, Bray, & Marsden, 1994). U.S. military forces operate worldwide, often living and working under stressful conditions and in close proximity while deployed to potentially hostile environments, or conducting sustained high-tempo operations in response to international events. Possible negative social behaviors that occur as a result of a military-wide smoking cessation effort could impact troop interactions and morale, and affect overall force readiness (Sommese & Patterson, 1995). For example, the cohesiveness of an entire flight crew could be adversely affected by an irritable aircraft commander who is required to abstain from smoking. The resulting lack of crew coordination as a result of the irritability or decreased attentiveness of essential crewmembers who are experiencing symptoms following nicotine cessation could jeopardize the safety and mission effectiveness of that aircraft and other units operating in its vicinity (Sommese & Patterson, 1995). Therefore, changes in aggressive behaviors that occur among individuals and groups, as a result of smoking cessation, merit investigation. The results of the present experiment suggest that nicotine cessation will not result in a rebound effect on aggressive behaviors. However, because nicotine dependence and withdrawal were not measured in the present study as noted above, future studies should evaluate aggressive behaviors during withdrawal following smoking cessation.

The results of the present experiment also suggest that nicotine or nicotine

analog drugs may be useful clinically for the treatment of conduct disorder or impulse-control disorders. A controlled form of nicotine administration, such as the nicotine transdermal patch or nicotine sublingual tablets, could be used to determine nicotine's effectiveness in reducing problematic behaviors that occur during these disorders. Follow-up studies conducted using samples of adolescents or adults screened for these conditions may determine nicotine's potential clinical use.

In conclusion, the present experiment revealed that nicotine reduced aggressive behaviors of rats when measured in a social interaction paradigm, especially rats that lived in single-housed conditions. These findings add to the literature regarding behavioral effects of nicotine, and suggest future studies and clinical implications.

Table 1: Correlations of Social, Exploratory, and Other Behaviors of During-Phase Animals

Correlations

Pearson Correlation

	BOLUS	BOX	FOLLOW	FREEZE	GROMSLF	MOVE	REAR	SNIFOTH	TOUCH	WRESTLE
BOLUS	1.000	-.216	-.361*	.339*	.046	.269	-.158	-.283	-.176	-.209
BOX	-.216	1.000	.106	-.356*	-.191	.061	.090	.243	.263	.478**
FOLLOW	-.361*	.106	1.000	-.410**	-.286*	-.087	.184	.519**	.498**	.378**
FREEZE	.339*	-.356*	-.410**	1.000	.285*	.234	-.376**	-.493**	-.350*	-.441**
GROMSLF	.046	-.191	-.286*	.285*	1.000	-.086	-.483**	-.290*	-.452**	-.413**
MOVE	.269	.051	-.087	.234	-.086	1.000	-.120	-.499**	.299*	.121
REAR	-.158	.090	.184	-.376**	-.483**	-.120	1.000	.091	.307*	.168
SNIFOTH	-.283	.243	.519**	-.493**	-.290*	-.499**	.091	1.000	.307*	.171
TOUCH	-.176	.263	.498**	-.350*	-.452**	.299*	.307*	.307*	1.000	.414**
WRESTLE	-.209	.478**	.378**	-.441**	-.413**	.121	.168	.171	.414**	1.000

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2: Correlations of Social, Exploratory, and Other Behaviors of Cessation-phase Animals

Correlations

Pearson Correlation

	BOLUS	BOX	FOLLOW	FREEZE	GROMSLF	MOVE	REAR	SNIFOTH	TOUCH	WRESTLE
BOLUS	1.000	.092	-.045	.222	.098	.277	-.072	-.124	-.088	-.057
BOX	.092	1.000	.057	-.224	-.020	.047	-.050	.012	.067	.633**
FOLLOW	-.045	.057	1.000	-.336*	-.509**	.033	-.173	.669**	.354*	.282
FREEZE	.222	-.224	-.336*	1.000	.266	.110	-.171	-.265	-.164	-.166
GROMSLF	.098	-.020	-.509**	.266	1.000	-.205	-.270	-.194	-.348*	-.231
MOVE	.277	.047	.033	.110	-.205	1.000	-.060	-.353*	.350*	.223
REAR	-.072	-.050	-.173	-.171	-.270	-.060	1.000	-.464**	.074	-.055
SNIFOTH	-.124	.012	.669**	-.265	-.194	-.353*	-.464**	1.000	-.034	.041
TOUCH	-.088	.067	.354*	-.164	-.348*	.350*	.074	-.034	1.000	.160
WRESTLE	-.057	.633**	.282	-.166	-.231	.223	-.055	.041	.160	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3: Results of Analyses of Social Behaviors

	Touch (*TCH*)	Sniff Other (*SNO*)	Follow
During Administration	**ME for Housing** *Single > Group* **ME for Sex** *Males > Females* **Sex x Housing** *Males↗ TCH in S-H,* *Males = Females in G-H* **Drug x Housing** (p=0.07) *Nicotine ↗ TCH in S-H,* *Nicotine ↘ TCH in G-H*	**ME for Drug** *Saline > Nicotine* **ME for Housing** *Single > Group* **Drug x Housing** *Nicotine ↘ SNO in G-H,* *Nicotine = Saline in S-H* **Sex x Housing** (p=0.077) *Females↘ SNO in G-H,* *Males = Females in S-H*	**ME for Housing** *Single > Group*
During Cessation		**ME for Drug** *Saline > Nicotine* **ME for Housing** *Single > Group* **ME for Sex** *Males > Females*	**ME for Housing** *Single > Group*
Males, During	**ME for Housing** *Single > Group*	**ME for Drug** (p=0.06) *Saline > Nicotine* **ME for Housing** *Single > Group* **Drug x Housing** *Nicotine ↘ SNO in G-H,* *Nicotine = Saline in S-H*	**ME for Housing** *Single > Group*
Females, During	**ME for Housing** *Single > Group* (p=0.072) **Drug x Housing** *Nicotine ↗ TCH in S-H,* *Nicotine ↘ TCH in G-H*	**ME for Housing** *Single > Group*	
Males, Cessation	**ME for Housing** *Single > Group*		**ME for Housing** *Single > Group*
Females, Cessation		**ME for Drug** *Saline > Nicotine* **ME for Housing** *Single > Group*	**ME for Housing** *Single > Group*
Males, During, Single			
Females, During, Single			
Males, During, Group	**ME for Drug** *Saline > Nicotine*	**ME for Drug** *Saline > Nicotine*	
Females, During, Group	**ME for Drug** *Saline > Nicotine*	**ME for Drug** *Saline > Nicotine*	**ME for Drug** *Saline > Nicotine*
Males, Cessation, Single			
Females, Cessation, Single			
Males, Cessation, Group			
Females, Cessation, Group		**ME for Drug** *Saline > Nicotine*	

Table 4: Results Analyses of Other Behaviors

	Bolus	Groom Self (*GS*)	Aggression
During Administration	**ME for Housing** *Group > Single*	**ME for Drug** *Nicotine > Saline* **ME for Housing** *Group > Single* **ME for Sex** *Males > Females* **Sex x Housing** (p=0.079) *Males ↗ GS in G-H,* *Males = Females in S-H*	**ME for Drug** *Saline > Nicotine* **ME for Housing** *Single > Group*
During Cessation	**ME for Sex** *Males > Females*	**ME for Housing** *Group > Single*	**ME for Housing** (p=0.092) *Single > Group*
Males, During		**ME for Housing** *Group > Single*	**ME for Housing** *Single > Group*
Females, During		**ME for Housing** *Group > Single* **Drug x Housing** *Nicotine ↗ GS in G-H,* *Nicotine = Saline in S-H*	**ME for Drug** (p=0.068) *Saline > Nicotine* **ME for Housing** *Single > Group*
Males, Cessation		**ME for Housing** *Group > Single*	
Females, Cessation	**ME for Housing** *Single > Group*	**ME for Housing** *Group > Single*	
Males, During, Single			**ME for Drug** (p=0.092) *Saline > Nicotine*
Females, During, Single			**ME for Drug** (p=0.054) *Saline > Nicotine*
Males, During, Group			
Females, During, Group			
Males, Cessation, Single		**ME for Drug** *Nicotine > Saline*	
Females, Cessation, Single			
Males, Cessation, Group	**ME for Drug** *Nicotine > Saline*		
Females, Cessation, Group			

Table 5: Results of Analyses of Exploratory Behaviors

	Freeze	Move (*MV*)	Rear (*RR*)
During Administration	**ME for Drug** *Nicotine > Saline* **ME for Housing** *Group > Single*	**ME for Drug** *Nicotine > Saline* **Sex x Housing** *Males ↗ MV in S-H,* *Males = Females in G-H* **Drug x Housing** (p=0.063) *Nicotine ↗ MV in G-H,* *Nicotine = Saline in S-H*	**ME for Drug** *Saline > Nicotine* **ME for Sex** *Females > Males* **Drug x Housing** (p=0.063) *Nicotine ↘ RR in G-H,* *Nicotine = Saline in S-H*
During Cessation		**ME for Housing** *Single > Group* **Sex x Housing** *Males ↗ MV in S-H,* *Males = Females in G-H*	**ME for Sex** *Females > Males* **Drug x Sex** (p=0.09) *Nicotine ↗ RR in Females,* *Nicotine = Saline in Males*
Males, During	**ME for Drug** *Nicotine > Saline*	**ME for Drug** *Nicotine > Saline* **ME for Housing** *Single > Group*	**ME for Drug** *Saline > Nicotine*
Females, During	**ME for Drug** (p=0.063) *Nicotine > Saline* **ME for Housing** *Group > Single* (p=0.058)	**ME for Drug** *Nicotine > Saline* **ME for Housing** *Group > Single* **Drug x Housing** *Nicotine ↗ MV in G-H,* *Nicotine = Saline in S-H*	**ME for Drug** *Saline > Nicotine* **Drug x Housing** (p=0.062) *Nicotine ↘ RR in G-H,* *Nicotine = Sal in S-H*
Males, Cessation		**ME for Housing** *Single > Group*	
Females, Cessation			**Drug x Housing** (p=0.091) *Nicotine ↗ RR in S-H,* *Nicotine = Saline in G-H*
Males, During, Single	**ME for Drug** (p=0.061) *Nicotine > Saline*		
Females, During, Single			
Males, During, Group	**ME for Drug** *Nicotine > Saline*	**ME for Drug** (p=0.09) *Nicotine > Saline*	**ME for Drug** (p=0.085) *Saline > Nicotine*
Females, During, Group	**ME for Drug** (p=0.076) *Nicotine > Saline*	**ME for Drug** *Nicotine > Saline*	**ME for Drug** *Saline > Nicotine*
Males, Cessation, Single			
Females, Cessation, Single			
Males, Cessation, Group			
Females, Cessation, Group			

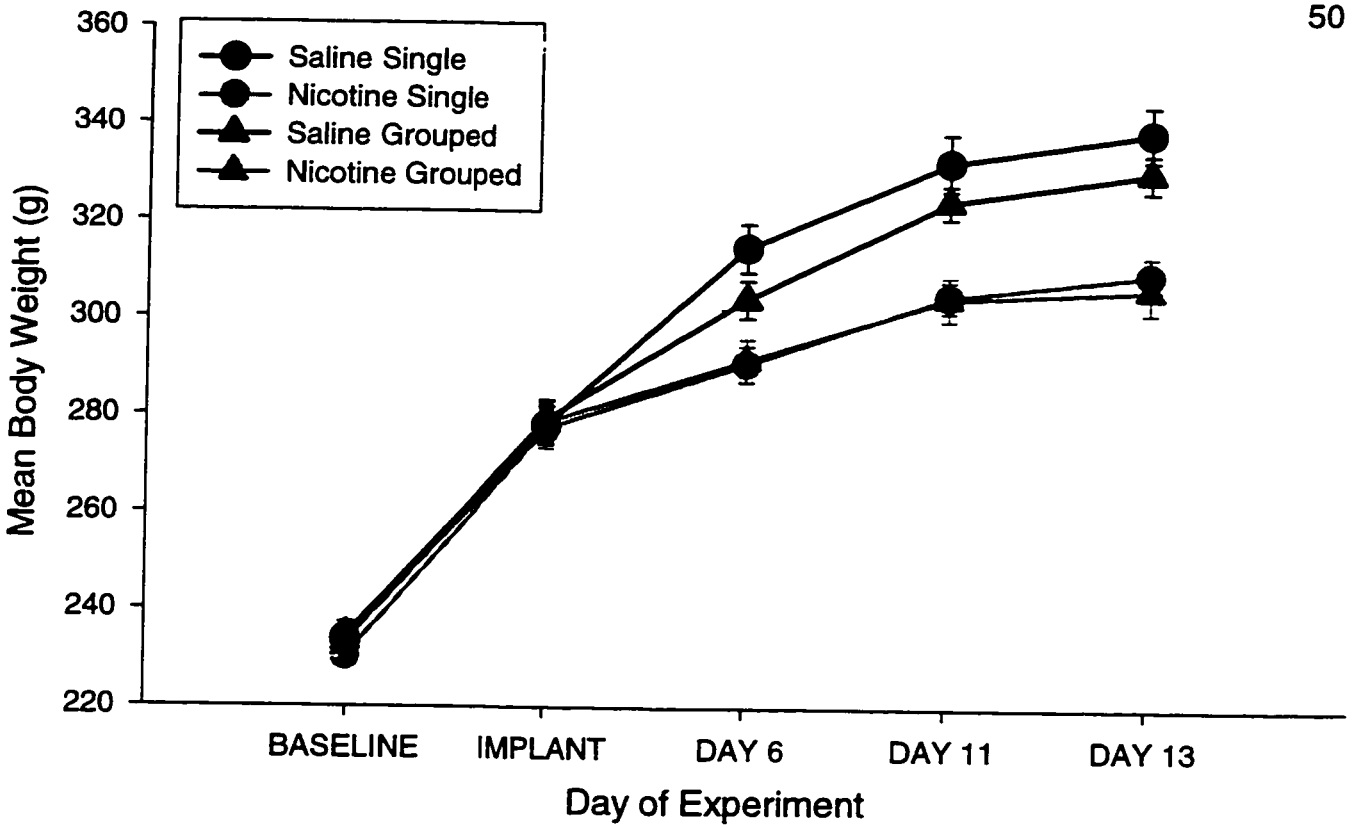


Figure 1: Effects of nicotine administration and cessation and housing condition on mean body weights of During-phase male rats.

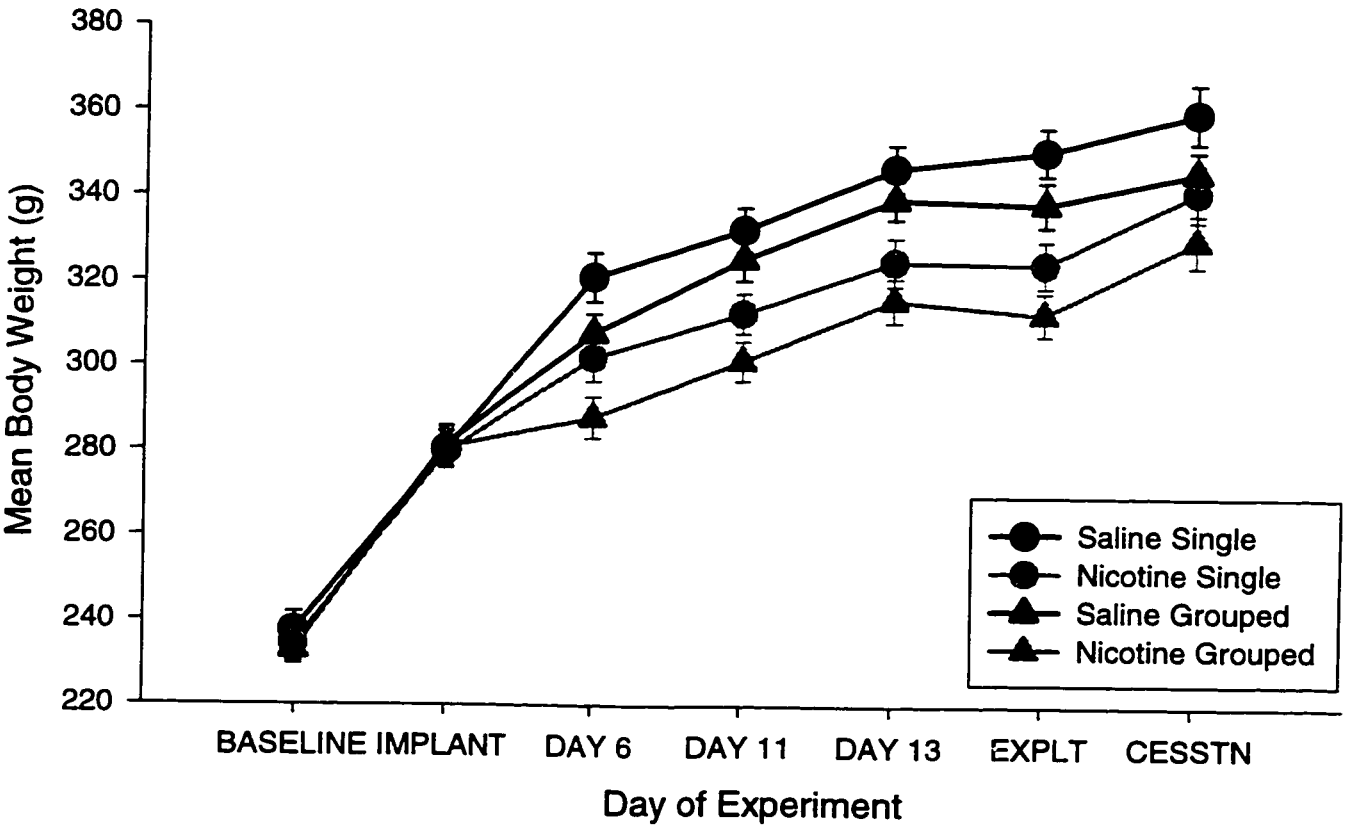


Figure 2: Effects of nicotine administration and cessation and housing condition on mean body weights of Cessation-phase male rats.

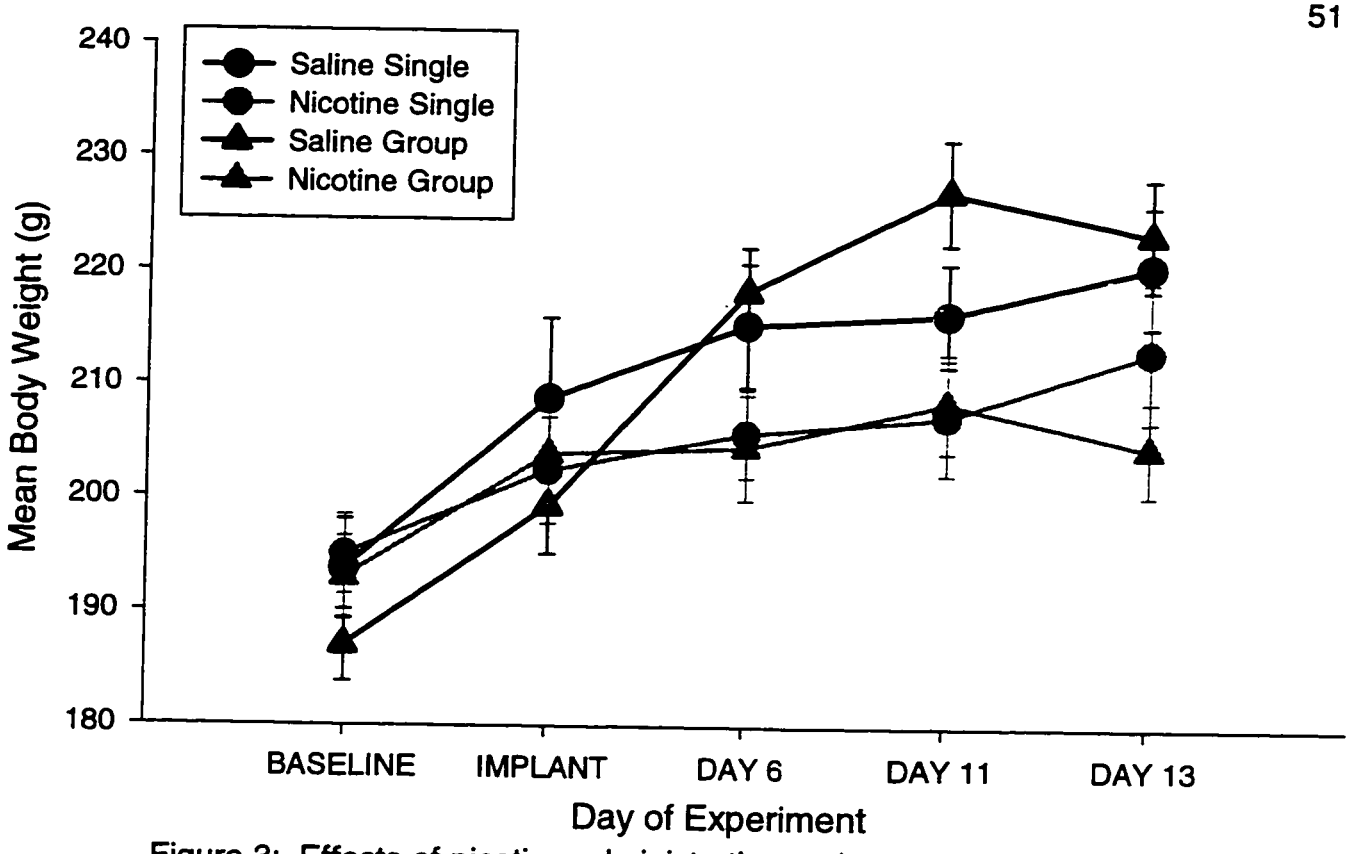


Figure 3: Effects of nicotine administration and cessation and housing condition on mean body weights of During-phase female rats.

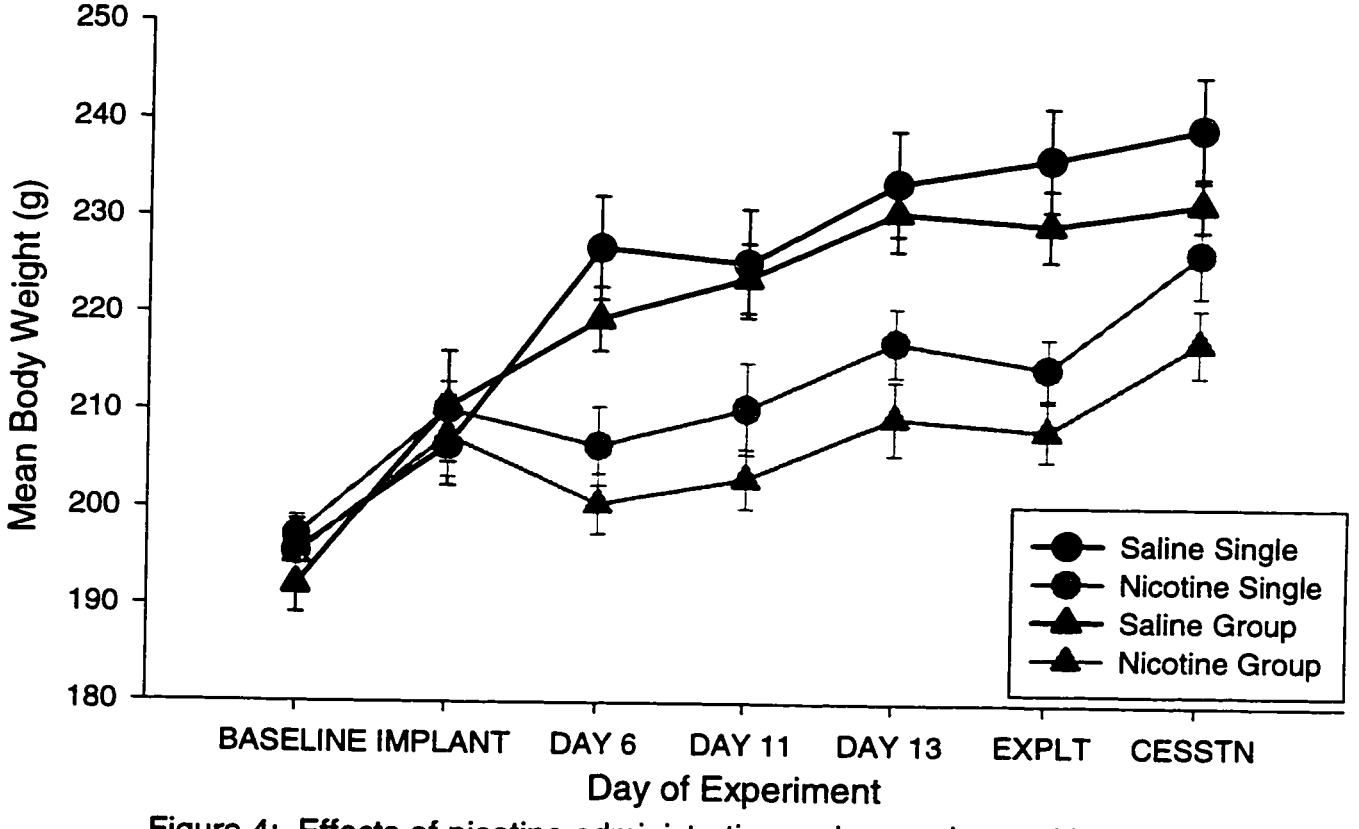


Figure 4: Effects of nicotine administration and cessation and housing condition on mean body weights of Cessation-phase female rats.

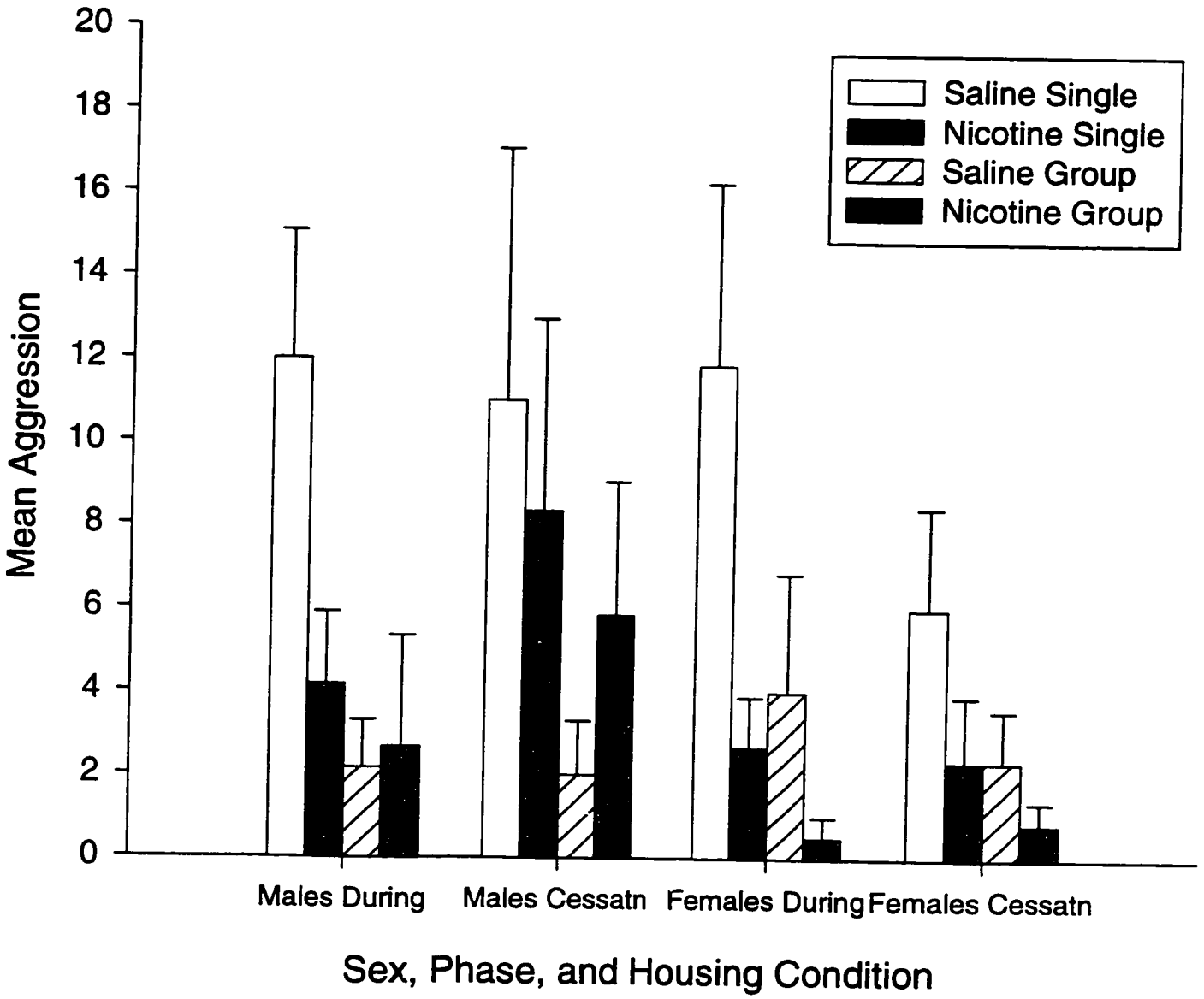


Figure 5: Effects of nicotine administration and cessation and housing condition on mean aggressive behaviors of male and female rats.

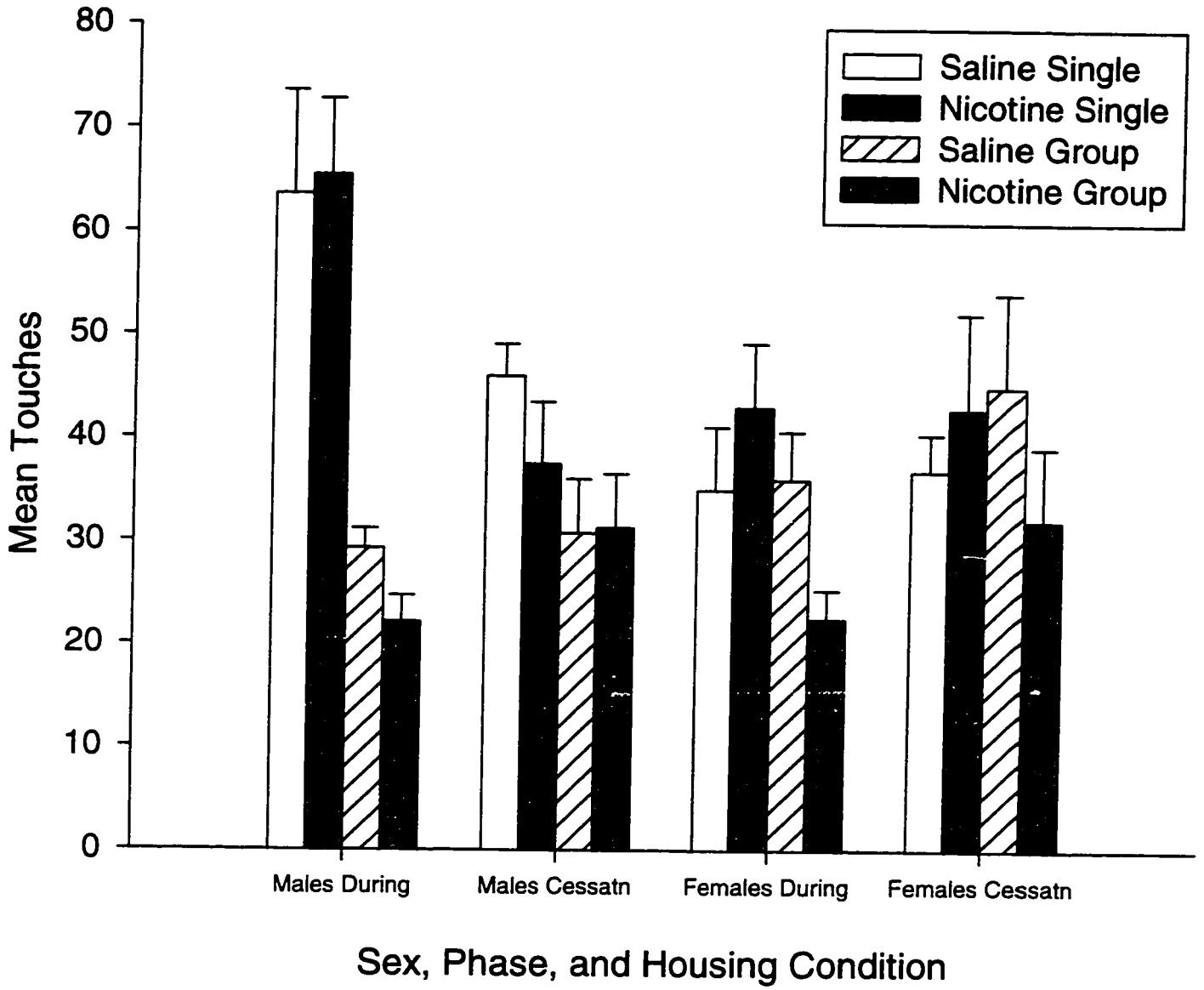


Figure 6: Effects of nicotine administration and cessation and housing condition on mean touching behaviors of male and female rats.

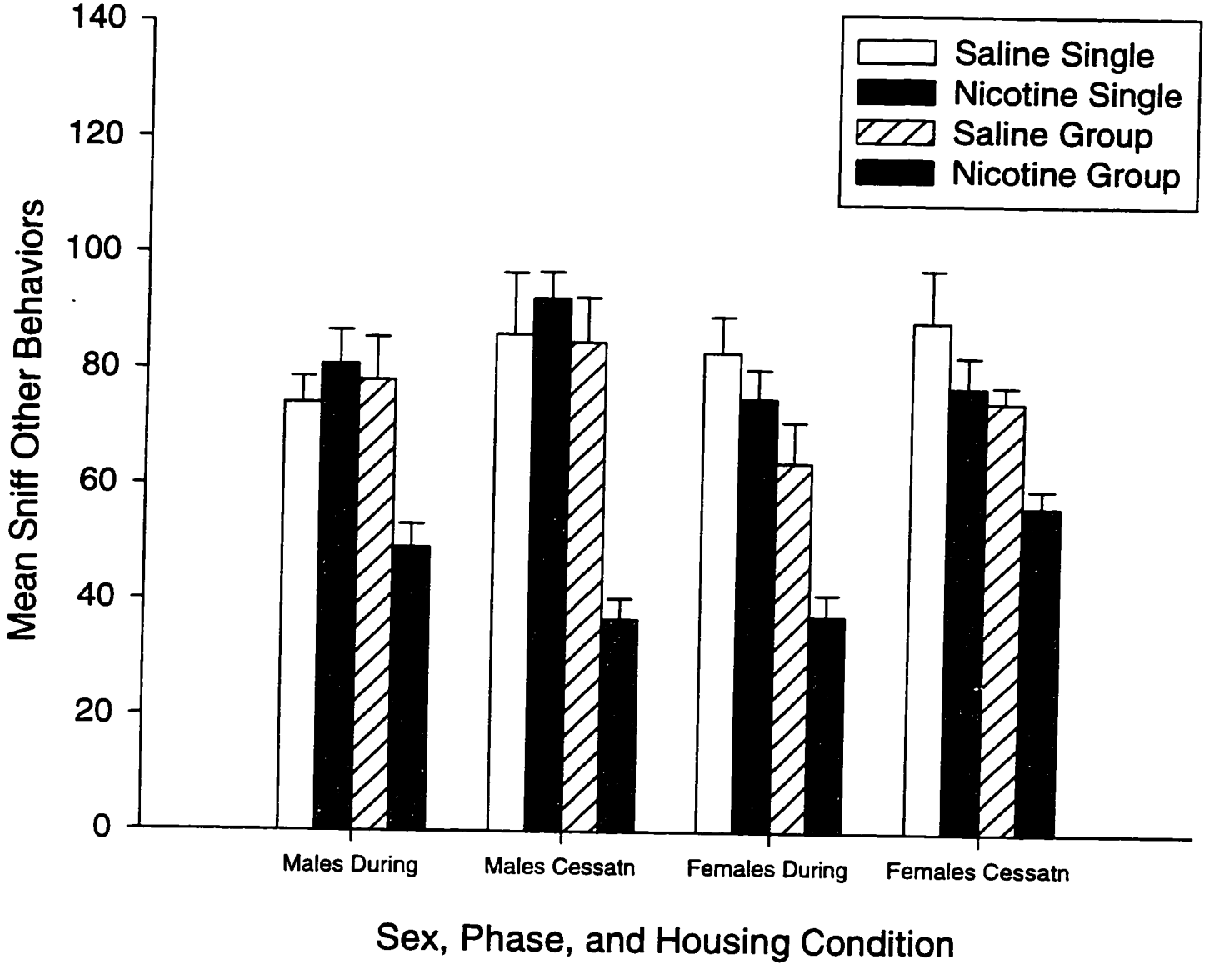


Figure 7: Effects of nicotine administration and cessation and housing condition on mean sniff other behaviors of male and female rats.

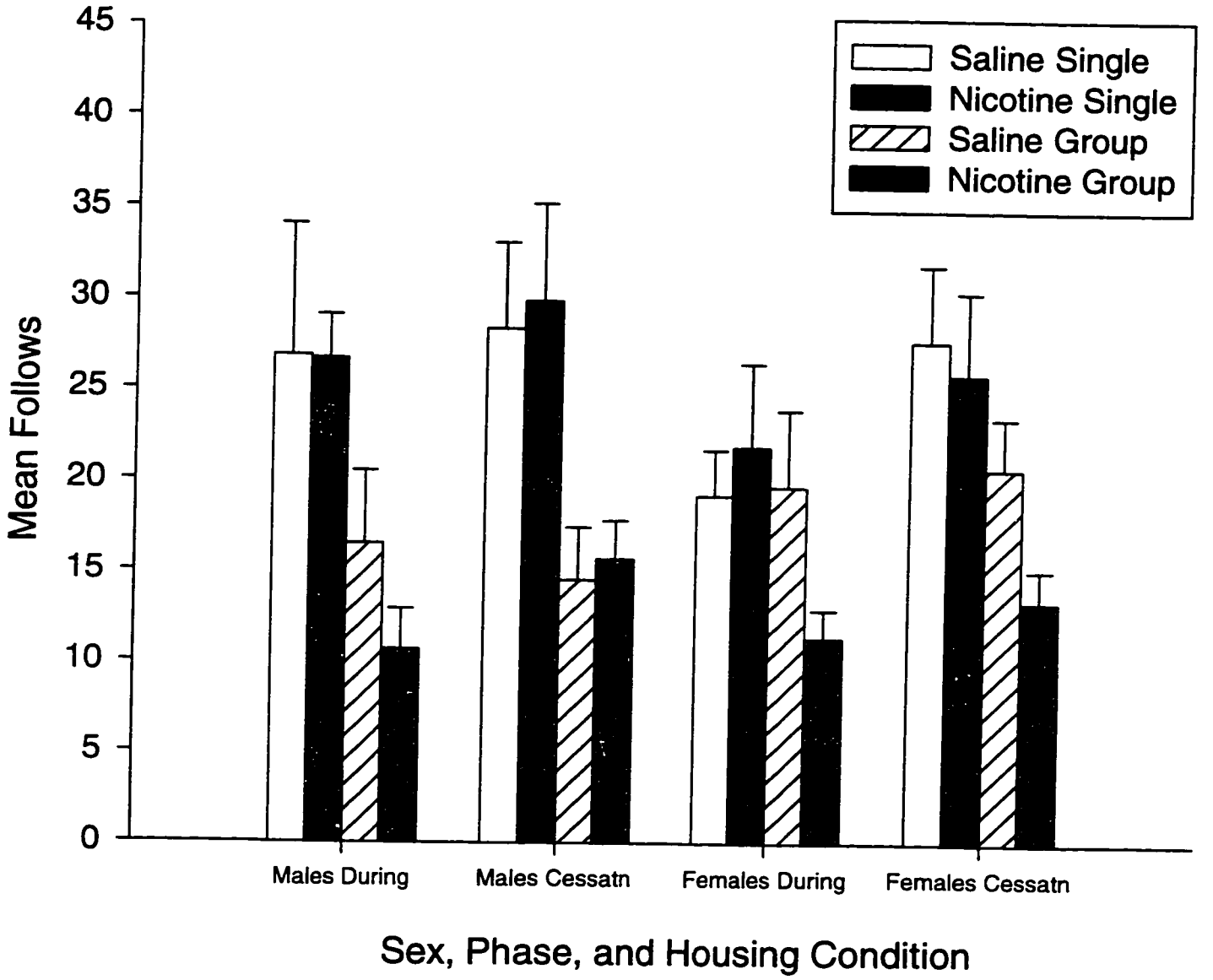


Figure 8: Effects of nicotine administration and cessation and housing condition on mean follow behaviors of male and female rats.

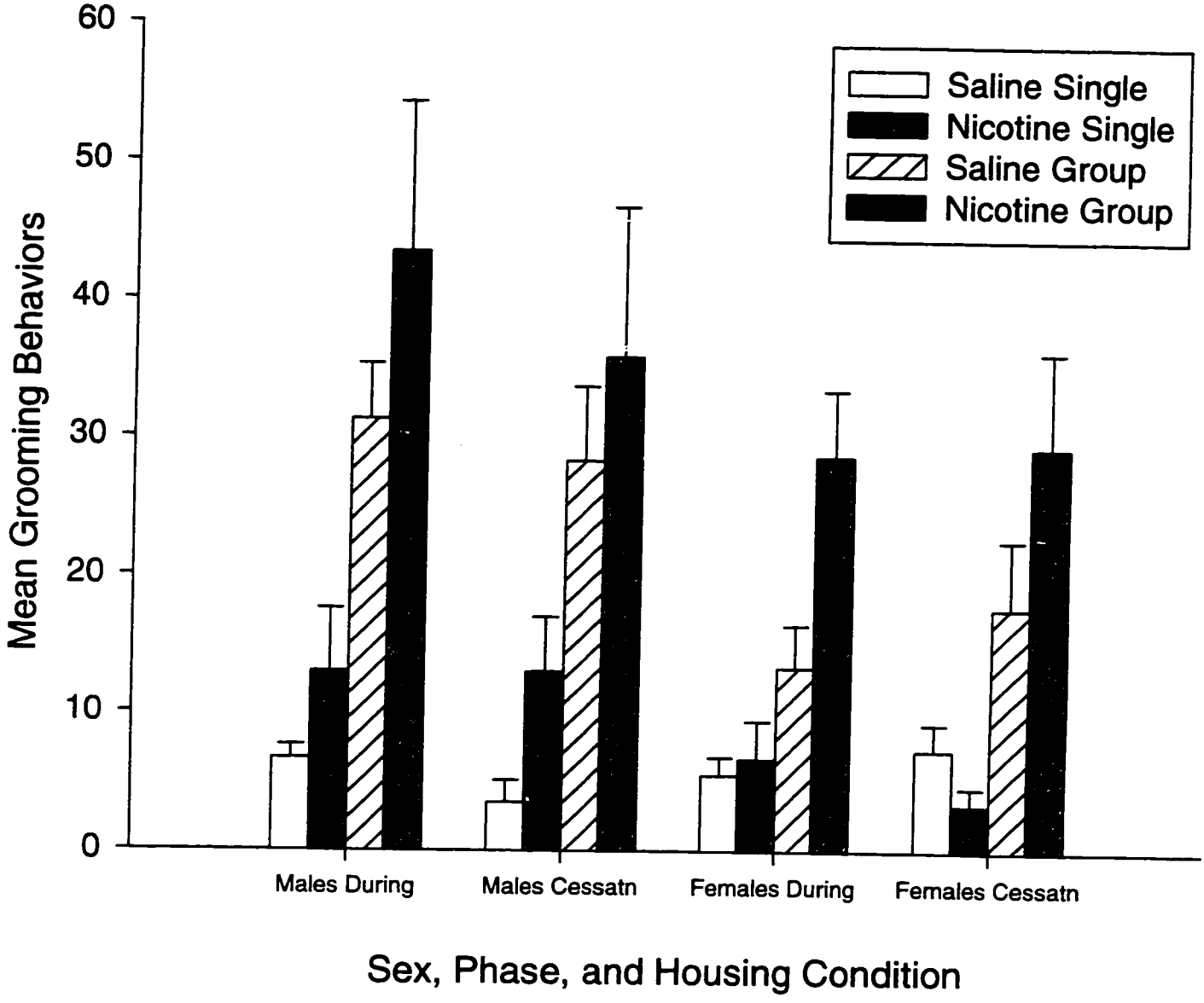


Figure 9: Effects of nicotine administration and cessation and housing condition on mean groom self behaviors of male and female rats.

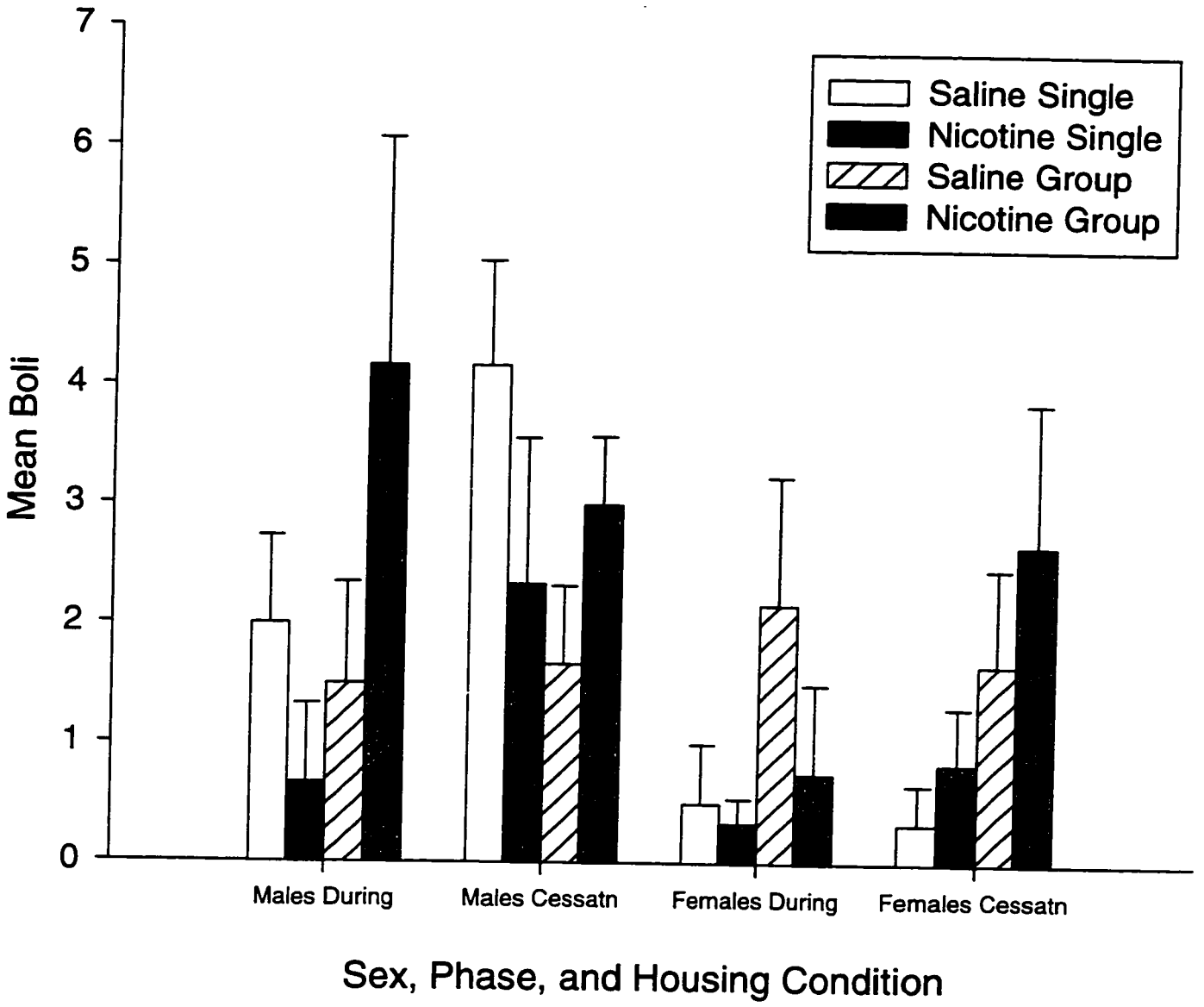


Figure 10: Effects of nicotine administration and cessation and housing condition on mean bolus behaviors of male and female rats.

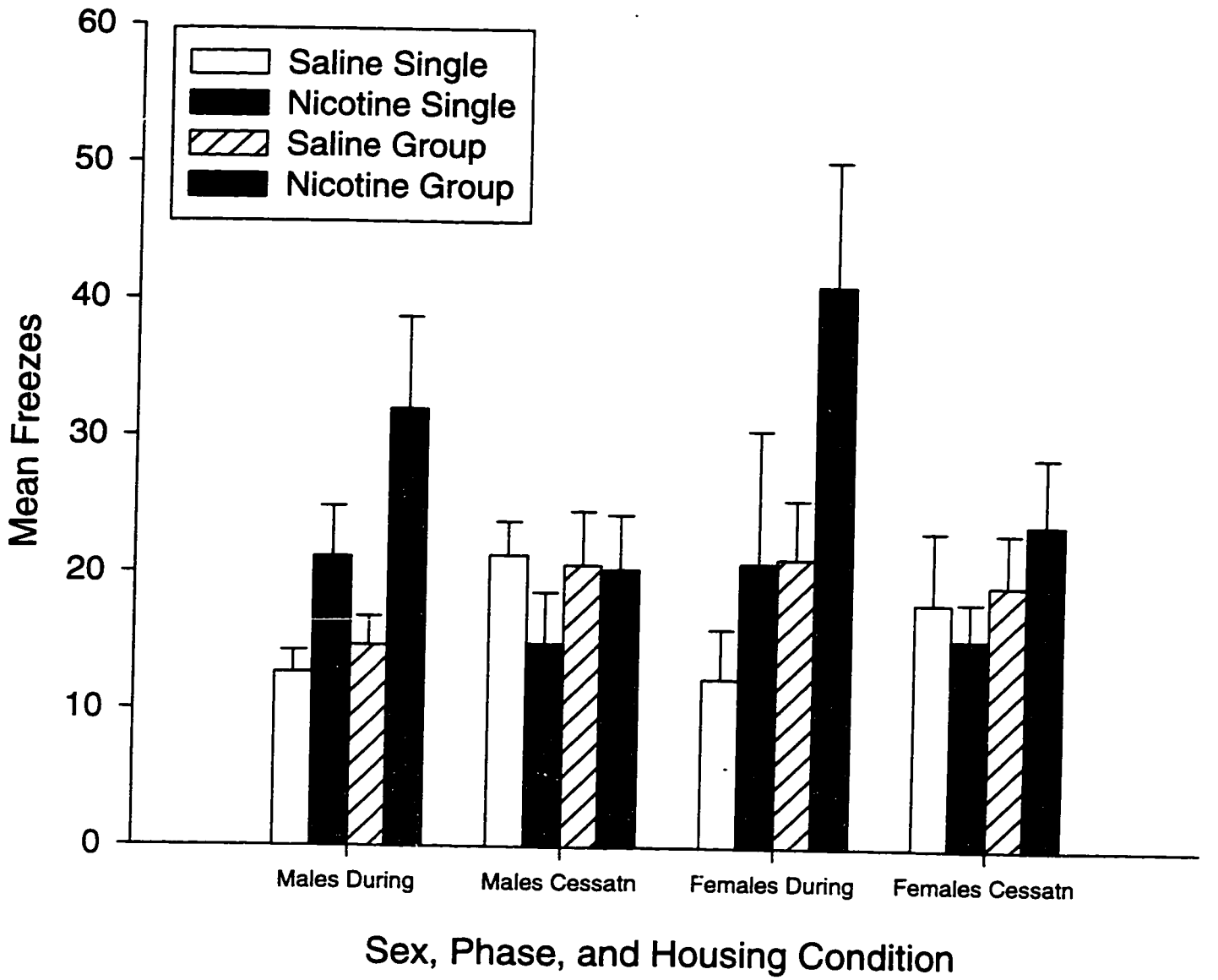


Figure 11: Effects of nicotine administration and cessation and housing condition on mean freeze behaviors of male and female rats.

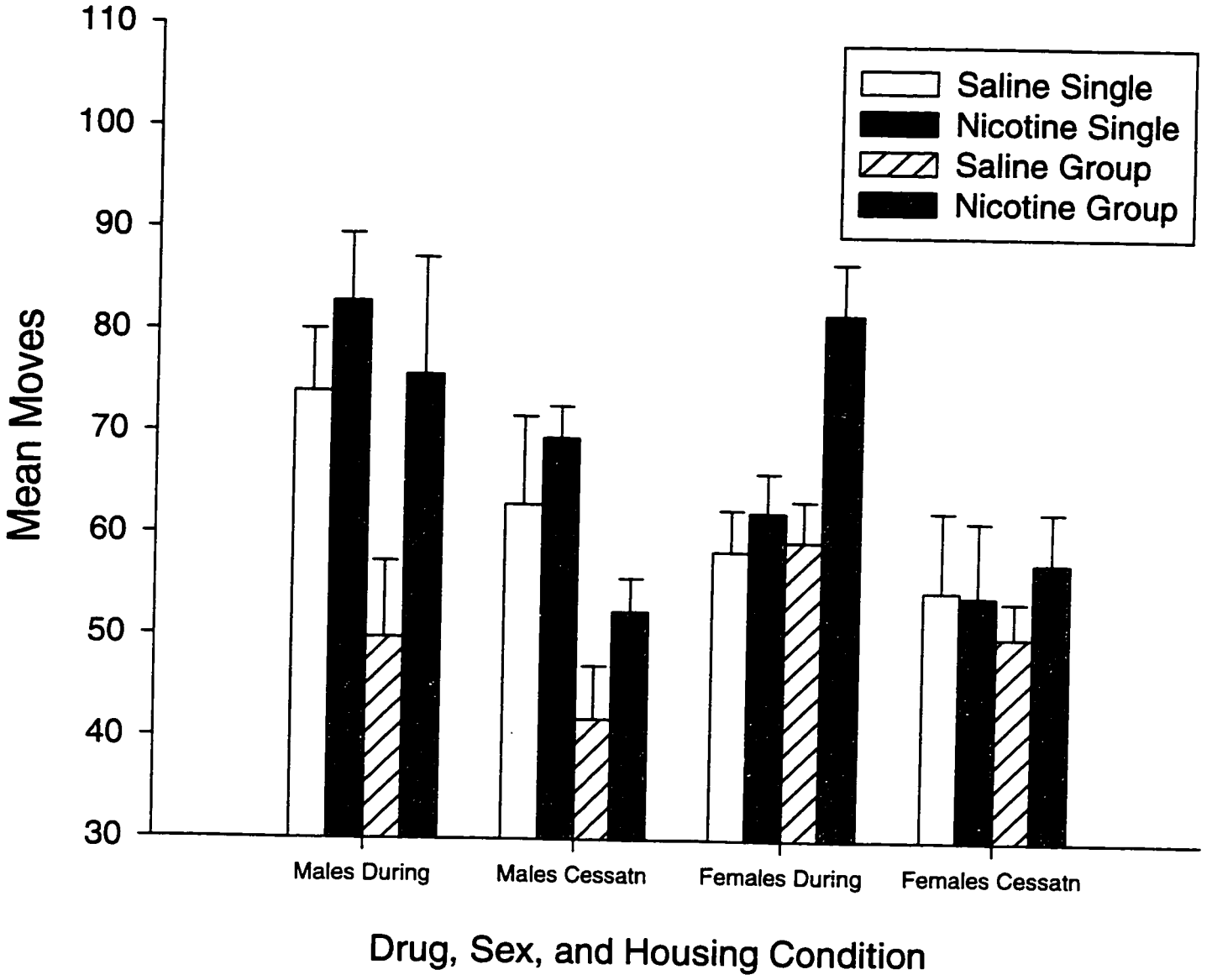


Figure 12: Effects of nicotine administration and cessation and housing condition on mean move behaviors of male and female rats.

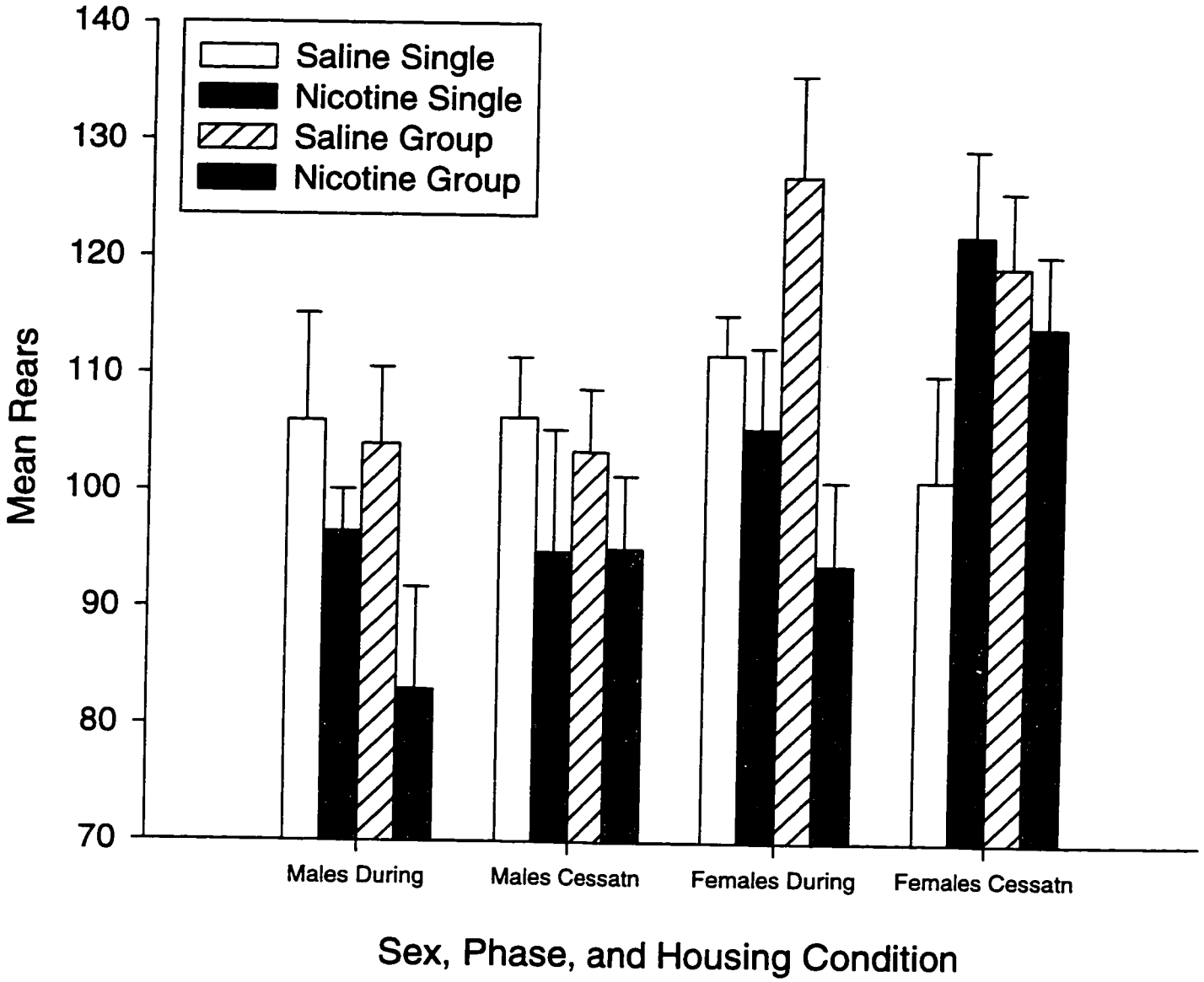


Figure 13: Effects of nicotine administration and cessation and housing condition on mean rear behaviors of male and female rats.

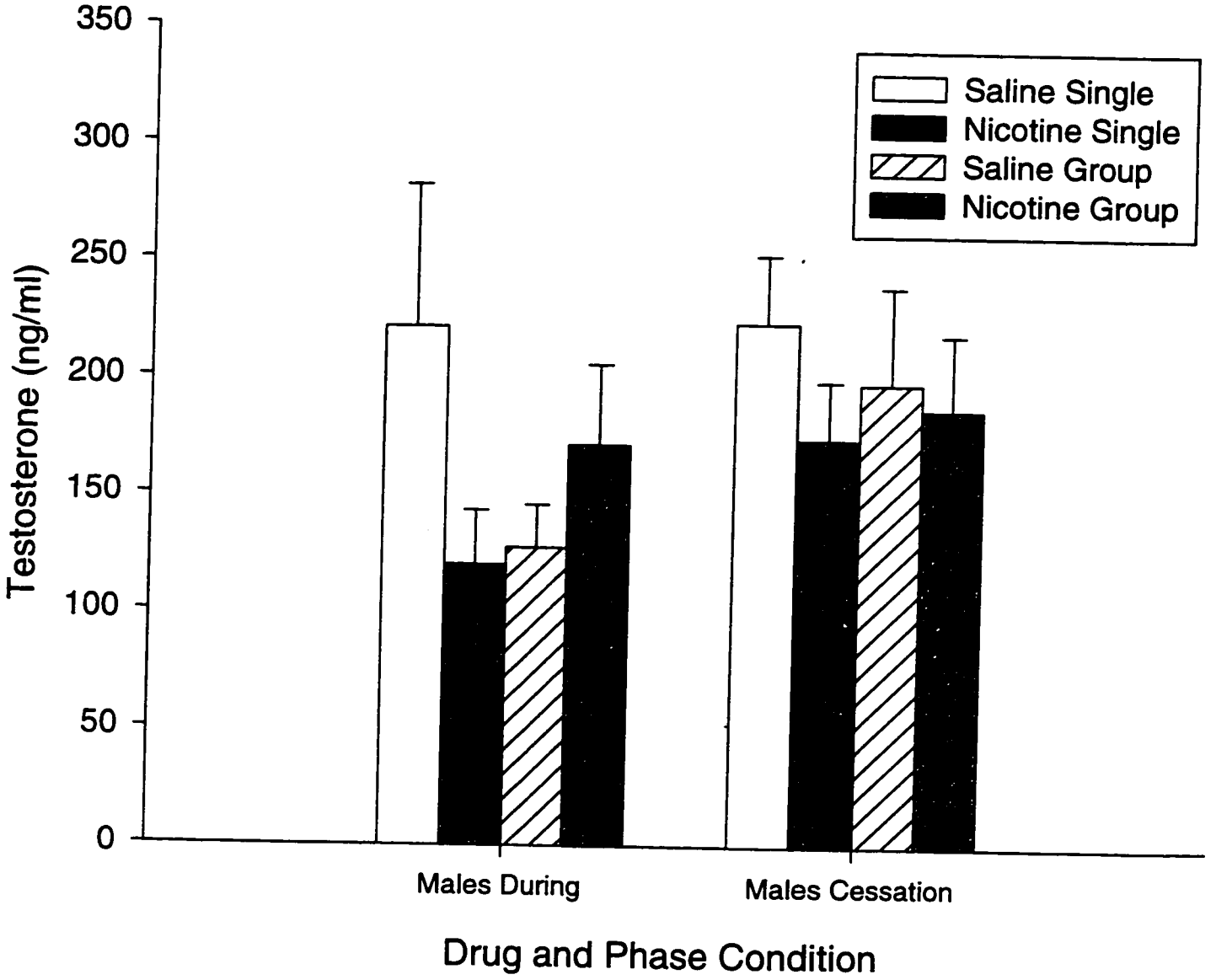


Figure 14: Effects of nicotine administration and housing condition on mean serum testosterone levels of male rats.

APPENDIX I

Examples of Rat Aggressive Behaviors:

A. Wrestling

B. Boxing

Example of Rat Aggressive Behavior: Wrestle

Example of Rat Aggressive Behavior: Boxing

APPENDIX II

Nicotine & Social Interaction Behavior Score Sheet and Scoring Protocol:

A. Behavior Score Sheet

B. Scoring Protocol

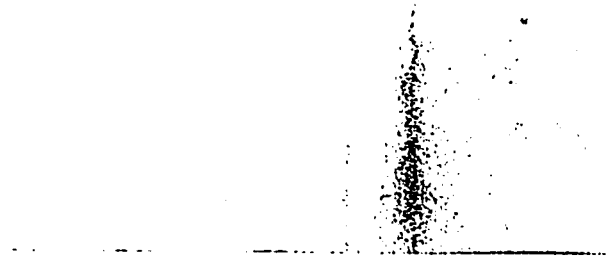
Nicotine & Social Interaction - Behavior Score Sheet

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Exploratory	Freeze																																								
	Sniff																																								
	Move																																							
	Move +Sniff																																							
	Rear																																							
Social	Touch																																							
	Follow																																							
	Sniff Other																																							
	Groom Oth.																																							
	Wrestle																																							
Other	Groom Self																																							
	Box/Bite																																							
	Eat																																							
	Submissive																																							
	Other																																							

Scorer Name: _____

Interaction #: _____ Animal Scored: _____

Date: _____ Time: _____



Nicotine & Social Interaction - Behavior Score Sheet

		41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Exploratory	Freeze																																								
	Sniff																																								
	Move																																							
	Move + Sniff																																							
	Rear																																							
Social	Touch																																							
	Follow																																							
	Sniff Other																																							
	Groom Oth.																																							
	Wrestle																																							
Other	Groom Self																																							
	Box/Bite																																							
	Eat																																							
	Submissive																																							
	Other																																							

Scorer Name: _____

Nicotine & Social Interaction - Behavior Score Sheet

		82	84	86	88	90	92	94	96	98	100	102	104	106	108	110	112	114	116	118	120
	Freeze																				
	Sniff																				
	Move																				
	Move + Sniff																				
	Rear																				
Social	Touch																				
Social	Follow																				
Social	Snifff Other																				
Social	Groom Oth.																				
Social	Wrestle																				
Other	Groom Self																				
Other	Box/Bite																				
Other	Eat																				
Other	Submissive																				
Other	Other																				

Scorer Name: _____

Interaction #: Animal Scored: _____ 3 of 5 Date: _____ Time: _____

Nicotine & Social Interaction - Behavior Score Sheet

		121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
	Freeze																																								
	Sniff																																								
	Move																																								
	Move + Sniff																																								
	Rear																																								
Social	Touch																																								
Social	Follow																																								
Social	Sniff Other																																								
Social	Groom																																								
Social	Wrestle																																								
Other	Groom Self																																								
Other	Box/Bite																																								
Other	Eat																																								
Other	Submissive																																								
Other	Other																																								

Scorer Name: _____

Interaction #: _____ Animal Scored: _____

Date _____ Time _____

Nicotine & Social Interaction - Behavior Score Sheet

		161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
Exploratory	Freeze																																								
	Sniff																																								
	Move																																							
	Move + Sniff																																							
	Rear																																							
Social	Touch																																							
	Follow																																							
	Sniff Other																																							
	Groom Oth.																																							
	Wrestle																																							
Other	Groom Self																																							
	Box/Bite																																							
	Eat																																							
	Submissive																																							
	Other																																							

Scorer Name: _____

Interaction #: _____ Animal Scored: _____ 5 of 5 Date _____ Time _____

Nicotine & Social Interaction Behavior Scoring Protocol

Goal:
Accurately & consistently score one animal's behavior every three seconds during ten minute interactions that have been recorded on tape.

Apparatus:

- Video Monitor
- Videocassette recorder
- Audiotape player
- NSI Behavior Score Sheets
- Pencils
- NSI Cue-card booklet

Animal Behaviors (from NSI Score Sheet):

Exploratory:

Freeze = The animal stops all movement, including total movement of the head.

Sniff = The animal has stopped locomotion, but continues to sniff the area around it. The animal may or may not move its head, but sniffing motions around the nostrils and whiskers are discernible and necessary for this score.

Move = The animal locomotes, but does not have head down and is not engaged in sniffing activities. The animal literally moves around the test arena.

Move + Sniff = The animal locomotes, but has its head down and is actively sniffing the area around it while locomoting.

Rear = The animal stops moving on four feet and shifts weight to back two feet. Typically, the animal rises up to investigate the sides of the test arena, but may also investigate other animal, or may be in the center of the arena.

Social:

Touch = The animal is in physical contact with the other animal. This may include touching tails, brushing hind-quarters, or sniffing other animal. Note: Sniffing other animal has its own coding category, below.

Follow = The animal locomotes in the same path as the other animal, with intention.

Sniff Other = The animal sniffs the other animal. This can include, but is not limited to sniffing the other's tail, genitalia, hindquarters, or facial/nose area.

Groom other = The animal engages in discernible grooming activity of the other animal.

Wrestle = The animal engages in wrestling activity with the other animal. Wrestling involves pushing with the front paws and/or upper body against the other animal. Also, score kicks with the back paw against the other animal as a "wrestle."

Other:

Groom self = The animal squats on hind legs and grooms itself with front paws.

Box/Bite = Boxing occurs when two animals are reared up on hind legs facing each other, and attempt to push each other with their front paws. Biting typically occurs when an animal moves around the side of another animal and bites it on the back of the neck. Some may nip the facial area or hindquarters of the other animal, however.

Eat = The animal can be seen eating something, usually feces.

Submissive = The rat submissive posture is flat on back, paws up, with the other animal typically on top.

Other = Behaviors not described above, such as the on top behavior.

Procedure:

1. Plug in monitor, VCR, and tape player. Turn on monitor and VCR.

2. Put videocasette in VCR, "NSI Metronome" audiotape in tape player.

3. Rewind audiotape to beginning.

4. Cue videotape to next interaction. Breaks in interactions are marked by NSI cue-card showing next interaction participant information.

5. When the next interaction is cued up, play tape until animals have been dropped into test arena, and lid has been placed on top. As hand is withdrawn, push pause.

6. Choose one of the animals to score behavior. Note tail marking of the animal. The NSI cue-card booklet can be used to help clear up questions on markings/identity of animals.

7. Fill in the information at the bottom of the first score sheet (self-explanatory).

8. Note the current behavior of animal (Most will freeze as lid is placed on top). Using a pencil, place a check-mark or slash in that behavior box in the first column of score sheet, marked at the top as "1".

9. Push "play" on the audiotape player. Have your pencil ready to use in one hand, and place your other hand on the VCR "play" button (but do not push it, yet).

10. When the taped voice says "play," push the play button on the VCR. Observe the animal you have chosen to score as it begins to move in real time.

11. When the taped voice says "two," name the behavior that the animal is currently engaged in, and place a mark in the box corresponding to that behavior under the column marked "2".

12. When the animals engage in an exploratory or other behavior, but are touching at the same time, place a mark in the "touch" box as well as the behavior named/marked.

13. Continue marking behaviors in this manner until the taped voice says "stop."

14. Watch and score three social interactions in the manner described above, and then take a ten-to-fifteen minute break before watching the next interaction(s).

APPENDIX III

Nicotine & Social Interaction Behavior Score Total Sheet

Scorer Name (1):_____ Scorer Name (2):_____

Interaction #:_____ Animal Scored (1):_____ Animal Scored (2):_____

Date(s):_____ Time(s):_____ Note(s):_____

Nicotine & Social Interaction - Behavior Totals Score Sheet

		1	2	3	4	5	T1	1	2	3	4	5	T2	Total
Exploratory	Freeze													
	Sniff													
	Move													
	Move +Sniff													
	Rear													
Social	Touch													
	Follow													
	Sniff Other													
	Groom Oth.													
	Wrestle													
Other	Groom Self													
	Box/Bite													
	Eat													
	Submissive													
	Other													

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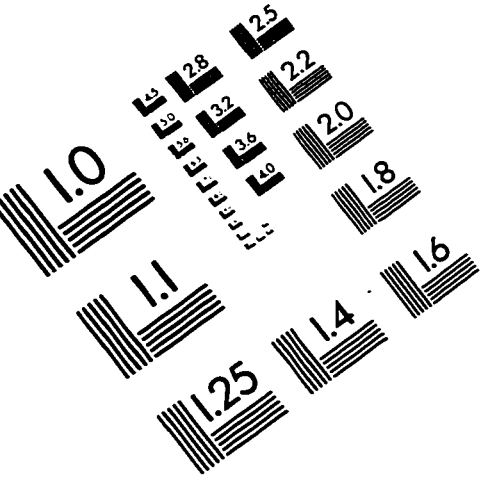
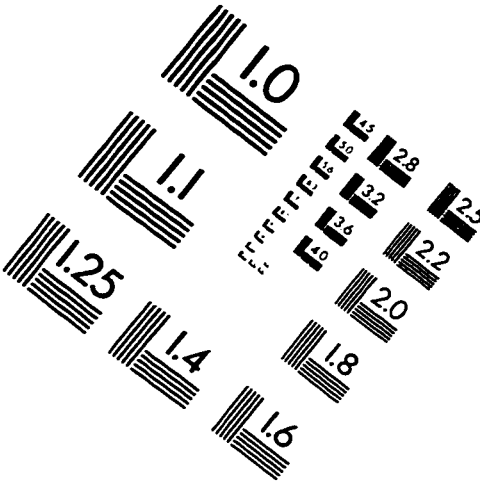
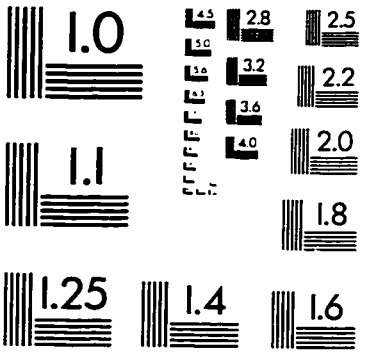
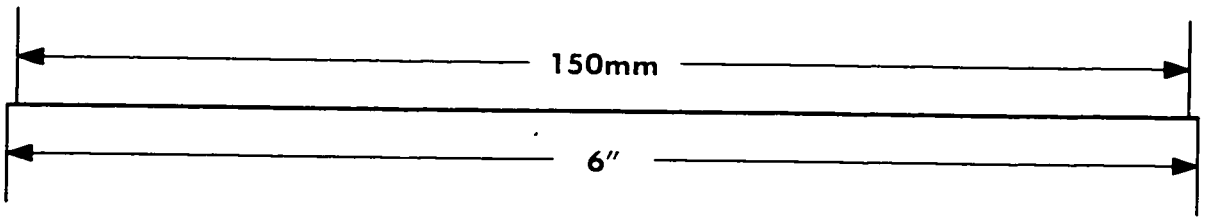
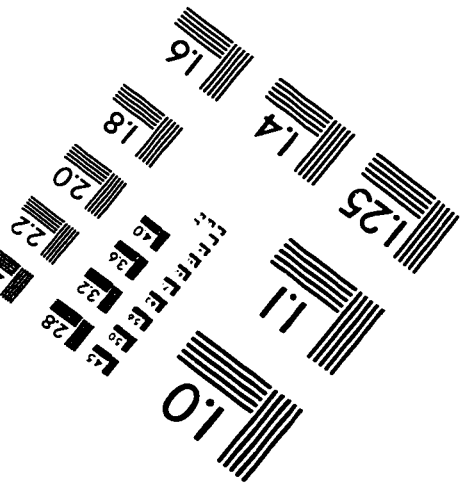
IMAGE EVALUATION TEST TARGET (QA-3)

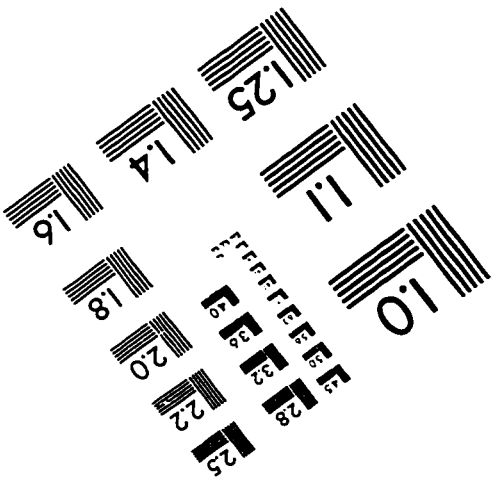












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